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## **A dendritic substrate for temporal diversity of cortical inhibition**

Annunziato Morabito, Yann Zerlaut, Dhanasak Dhanasobhon, Emmanuelle Berthaux, Alexandra Tzilivaki, Gael Moneron, Laurence Cathala, Panayiota Poirazi, Alberto Bacci, David A. Digregorio, et al.

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3 **Title: A dendritic substrate for temporal diversity of cortical inhibition**  
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37 **Abstract**

38 In the mammalian neocortex, GABAergic interneurons (INs) inhibit cortical networks in  
39 profoundly different ways. The extent to which this depends on how different INs process  
40 excitatory signals along their dendrites is poorly understood. Here, we reveal that the functional  
41 specialization of two major populations of cortical INs is determined by the unique association of  
42 different dendritic integration modes with distinct synaptic organization motifs. We found that  
43 somatostatin (SST)-INs exhibit NMDAR-dependent dendritic integration and uniform synapse  
44 density along the dendritic tree. In contrast, dendrites of parvalbumin (PV)-INs exhibit passive  
45 synaptic integration coupled with proximally enriched synaptic distributions. Theoretical analysis  
46 shows that these two dendritic configurations result in different strategies to optimize synaptic  
47 efficacy in thin dendritic structures. Yet, the two configurations lead to distinct temporal  
48 engagement of each IN during network activity. We confirmed these predictions with *in vivo*  
49 recordings of IN activity in the visual cortex of awake mice, revealing a rapid and linear  
50 recruitment of PV-INs as opposed to a long-lasting integrative activation of SST-INs. Our work  
51 reveals the existence of distinct dendritic strategies that confer distinct temporal representations  
52 for the two major classes of neocortical INs and thus dynamics of inhibition.

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57 **Main Text**

58 In the neocortex, fast synaptic inhibition originates from a rich diversity of inhibitory neurons  
59 (INs) that play a fundamental role in regulating cortical function by orchestrating network  
60 dynamics (1–3). Importantly, this IN diversity is associated with differential functional roles of  
61 specific INs within neuronal circuits (3, 4). The diverse IN functions are thought to result mainly  
62 from differences in connectivity profiles, specific biophysical properties, strength and dynamics  
63 of excitatory glutamatergic synapses that recruit GABAergic INs (5–10). However, INs primarily  
64 receive synaptic inputs along their dendrites, and it remains unclear whether distinct dendritic  
65 properties across different IN populations contribute to their specific activity profiles and thus  
66 computational roles in cortical circuit function.

67 Recent evidence suggests that IN dendrites can exhibit nonlinear integration of synaptic  
68 activity, similarly to PNs, thus increasing the computational power of INs (for a review see (5–8)).  
69 However, most research on dendritic operations in INs was limited to PV cells of either  
70 hippocampus (8–12) or cerebellum (13, 14). Indeed, it has been shown that oriens-alveus (O/A)-  
71 SST INs in the hippocampus exhibit significant differences in the density of voltage-gated  
72 potassium and sodium channels as compared to PV-INs (15), indicating that dendritic properties  
73 might vary between different populations of INs. Yet, it is presently uncertain whether specificities  
74 in dendritic integration determine the functional properties of different populations of INs.

75 Among the different subtypes, PV- and SST-IN are the two main IN populations that provide  
76 inhibitory control of PN networks. They do so, however, using distinct dynamics. Fast-spiking,  
77 PV basket cells are phase-locked to fast oscillations, are highly responsive to increases in activity  
78 of excitatory inputs and regulate PN activity via perisomatic inhibition with millisecond-level  
79 precision (5, 16–18). In contrast, the activation of SST-INs requires bursts from single PNs or the  
80 firing of multiple PNs. Moreover, SST-INs fire with less precision, and inhibit PN dendritic  
81 segments over longer timescales (19–24). These distinct features are essential for cortical network  
82 properties, including emergence of oscillatory rhythms and facilitating learning through dendritic  
83 inhibition and gating synaptic plasticity (18, 23, 25–28). However, whether differences in dendritic  
84 integration properties play a role in determining the temporal dynamics in the recruitment of PV-  
85 and SST-INs remains largely unexplored. Here, we demonstrate that PV- and SST-INs employ  
86 distinct strategies for integrating excitatory inputs on their dendrites. These strategies govern the  
87 firing dynamics of each interneuron subclass, differently affect the encoding of sensory inputs by  
88 cortical networks, and thereby define their specific functional roles within cortical networks.

89

90 *PV- and SST-INs possess distinct dendritic integration properties.*

91

92 To investigate synaptic integration in dendrites of neocortical INs, we performed whole-  
93 cell, current-clamp recordings of PV and SST-INs in layer 2/3 of primary somatosensory cortex in  
94 adult mice and used two-photon glutamate uncaging to mimic synapse activation (13, 29); **Fig.**  
95 **1A-D**). We monitored somatic voltage responses to both single synapse activation and to multiple  
96 quasi-synchronous input sequences. To assess deviations from linear synaptic integration, we  
97 compared the arithmetic sum (which represents the linear prediction) of individual photolysis-  
98 evoked excitatory postsynaptic potentials (pEPSPs) with actual responses evoked by quasi-  
99 simultaneous (0.12 ms inter-pulse interval) activation of all locations (**Fig. 1A,C**). We found an  
100 overall sublinear integration mode in PV-INs dendrites (**Fig. 1A,B**) and an overall supralinear  
101 integration in SST-INs (**Fig. 1C,D**). Such a difference was also observed for different time

102 intervals between active inputs (**Fig. S1**). The supralinear integration observed in SST-INs was not  
103 significantly different from the values obtained in pyramidal neurons (PNs) (**Fig. 1D,E**).

104 In PNs, integration of glutamatergic synaptic inputs along individual dendritic branches  
105 has been shown to be strongly dependent on the presence of synaptic NMDARs (30–32). We  
106 explored whether variations in dendritic integration were associated with differences in the relative  
107 contribution of NMDA and AMPA receptors (AMPA) at excitatory synapses onto PV- and SST-  
108 INs. Using voltage-clamp recordings and extracellular stimulation, we observed that SST-INs  
109 exhibit a higher NMDA-to-AMPA ratio when compared to PV-INs (**Fig. 1F, G**). This difference  
110 was confirmed by paired recordings from unitary synaptic connections between individual L2/3  
111 PNs and PV- or SST-INs (**Fig. S2A,B**). Furthermore, by examining NMDAR-dependent miniature  
112 EPSCs at -30 mV (isolated using the NMDAR blocker D-AP5, 100  $\mu$ M), we found that the marked  
113 NMDA/AMPA differences between PV- and SST-INs was predominantly due to a much weaker  
114 expression of the NMDAR-mediated component in PV-INs (**Fig. S2C,D**). To test whether the  
115 enhanced levels of synaptic NMDARs in SST-INs defined its supralinear integration properties,  
116 we measured subthreshold input-output function of L2/3 SST-INs in the presence of  
117 pharmacological blockade (MK-801 and D-AP5) or upon genetic removal (GluN1 KO) of  
118 NMDARs. In both conditions, supralinear integration was abolished and integration of quasi-  
119 synchronous inputs resulted in compound EPSPs smaller than the arithmetic linear sum (**Fig. 1H**).

120 Sublinear integration observed in PV-INs is compatible with a decrease in driving force  
121 due to large local dendritic depolarizations as observed in cerebellar stellate (13) and Golgi cells  
122 (14), as well as in hippocampal and L5 prefrontal cortex PV interneurons, where both supralinear  
123 and sublinear branches coexist (11, 12). To investigate whether such an effect could explain  
124 neocortical PV-IN integration, we analyzed a reduced theoretical model implementing cable  
125 theory in a simplified morphology ((33), **Fig. 1I**). Model parameters were constrained to match  
126 our electrophysiological and morphological measurements (see **Methods**). This simple passive  
127 dendritic model recapitulated sublinear integration in PV INs because of high local input resistance  
128 (**Fig. 1J**, top). The model also predicted that input-output curves strongly relied on spatial location  
129 of excitatory synapses, with sublinear integration being particularly robust at distal locations (**Fig.**  
130 **1J**, bottom). To test this, we performed synaptic integration experiments separately at proximal  
131 and distal locations (**Fig. 1K**, <40 $\mu$ m and >100 $\mu$ m from the soma respectively). Uncaging laser  
132 power was adjusted to mimic average EPSPs amplitude at the corresponding specific dendritic  
133 locations (**Fig. S3**). In accordance with the model predictions, we observed that PV-INs exhibited  
134 strong sublinear responses when dendrites were stimulated distally. In contrast, dendritic  
135 integration was mostly linear when dendrites were stimulated proximally (**Fig. 1K,L**). Conversely,  
136 responses of SST-IN dendrites were supralinear, regardless of dendritic location (**Fig. 1L**).  
137 Importantly, differences in dendritic integration between PV- and SST-INs could not be  
138 attributable to differences in dendritic diameter along their length, as both cell types exhibited  
139 similar dendritic diameter profiles (**Fig. S4**). Overall, these results indicate that the dendrites of  
140 PV- and SST-INs have different dendritic integration properties. PV-INs display dendritic  
141 integration gradients along the dendritic tree with strong distal sublinear integration, compatible  
142 with passive integration in thin dendrites (11, 13). On the other hand, SST-INs display NMDAR-  
143 dependent supralinear integration along the entire length of their dendrites, thus providing effective  
144 excitatory input integration despite cable filtering properties of thin dendrites.

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*Synaptic integration of multivesicular release is different between PV- and SST-INs.*

148 Previous studies showed that a consequence of nonlinear integration is the distance-  
149 dependent alteration in short-term plasticity (13). We therefore hypothesized that the distinct  
150 dendritic integration properties could result in differential processing of short-term presynaptic  
151 plasticity by PV- and SST-IN dendrites. This effect becomes especially significant if multiple  
152 release events (*i.e.*, quantal content greater than one) occur at single points of contact and require  
153 integration in close temporal and spatial proximity (13). We thus examined if glutamatergic  
154 synapses innervating PV- and SST-INs elicited multiple quantal release events per individual  
155 action potential. To address this question, we used the genetically-encoded glutamate sensor,  
156 iGluSnFR (34) to monitor glutamate release events from single L2/3 PN axonal boutons *in vitro*  
157 (**Fig. 2A**). iGluSnFR was expressed in either SST- or PV-INs. By loading individual L2/3 PNs  
158 with Alexa 594 via the whole-cell pipette, and utilizing two-photon imaging, we were able to  
159 identify putative points of contact between presynaptic axons and iGluSnFR expressing IN  
160 dendrites (**Fig. 2A,B**). To elicit glutamate release, we evoked action potentials in presynaptic L2/3  
161 PNs via current injection (**Fig. 2A**). We successfully identified specific functional synaptic  
162 contacts, enabling the detection of glutamate release events even within individual recording  
163 sweeps (**Fig. 2B-C**).

164 We separated successful and failed release events by comparing the amplitude of evoked  
165 changes in iGluSnFR fluorescence after each individual-evoked action potential with baseline  
166 values (see **Methods** and **Fig. S5**). Overall, as anticipated for low release probability synapses,  
167 failures were significantly more pronounced in inputs targeting SST-INs in comparison to those  
168 targeting PV-INs (**Fig. 2G,H,I**). These observations were consistent in experiments conducted  
169 with both 2 mM (**Fig. 2**) and 1.2 mM extracellular calcium concentrations (**Fig. S5**). In line with  
170 the higher number of failures, the coefficient of variation (CV) was greater in inputs onto SST-INs  
171 when compared to PV-INs (**Fig. 2C,D** and **Fig. S5**). The number of release sites (N) defines the  
172 maximum number of synaptic vesicles that could be released per action potential at individual  
173 synaptic locations (35). To estimate N, we performed optical fluctuation analysis (36, 37),  
174 assuming a binomial distribution of release events. We found that N was quite variable between  
175 individual points of contact, like previously reported in hippocampal INs (38), with a mean value  
176 per action potential close to 2 for both SST and PV-INs (**Fig. 2D** and **Fig. S5**). Overall, our results  
177 reveal that multivesicular release can occur at individual glutamatergic inputs in both cortical SST-  
178 and PV-INs.

179 To test how multivesicular release is integrated in dendrites of PV and SST-INs, we probed  
180 the integration of single and double-quanta at single dendritic locations along the dendrites of PV-  
181 and SST-INs, using quantal-size adjusted pEPSCs (**Fig. 2E,F**). Dendrites of SST-INs integrated  
182 double-quanta linearly all along the dendritic tree (**Fig. 2G,H**). This linear integration was also  
183 observed in current clamp configuration (**Fig. S6**). Conversely, PV-IN dendrites exhibited a  
184 progressive somatodendritic attenuation of double-quanta pEPSCs as expected for a passive cable  
185 (13) (**Fig. 2F-H**). This decrease in short-term plasticity (STP) was observed also while mimicking  
186 depressing inputs that represent the natural plasticity of glutamatergic inputs contacting PV-INs  
187 (**Fig. S6**). We subsequently tested the mechanism involved in the relatively linear double-quanta  
188 integration along SST-INs dendrites. Blocking NMDARs significantly reduced the ability of SST-  
189 IN dendrites to linearly detect two release events at distal dendritic locations (**Fig. 2I,J**). This effect  
190 was not further increased by blocking other voltage-gated conductances (**Fig. 2I,J**). In  
191 hippocampal PV-INs, activation of potassium channels was proposed to participate in sublinear  
192 integration of synaptic inputs (5, 39). However, blocking potassium channels with TEA did not  
193 reduce the sublinear integration of double quanta along dendrites in cortical PV-INs (**Fig. 2K,L**).

194 Our findings indicate that during synaptic transmission, the active dendritic properties of  
195 SST-INs contribute to the preservation of presynaptic STP. Conversely, in PV-INs, passive  
196 dendrites are expected to dampen STP, particularly for synaptic contacts located within more distal  
197 dendritic regions. Consistent with this hypothesis, unitary synaptic responses from paired  
198 recordings between L2/3 PNs and PV-INs exhibited a significant inverse relationship between the  
199 rise time of EPSCs (a proxy for input location) and short-term plasticity in PV-INs. Such  
200 correlation was absent in the unitary EPSCs between PNs and SST-INs (**Fig. S7**). Overall, these  
201 experiments suggest that interneuron-specific dendritic operations reduce short-term synaptic  
202 plasticity in PV-INs and maintain short-term facilitation in SST-INs. These properties may  
203 therefore modify the dynamic activity of PV and SST interneurons during network activity.

204

205 *Distinct spatial synaptic distribution along the dendrites of PV- and SST-INs.*

206

207 Our results so far support different dendritic integration properties in neocortical L2/3 PV-  
208 and SST-INs. How these operations contribute to integration of synaptic inputs is determined by  
209 the timing and location of active synapses. These patterns are partly controlled by the way synaptic  
210 inputs distribute along the dendritic tree of neurons. Previous work has shown that synapse density  
211 gradients can significantly alter dendritic responses (40, 41) but whether synapse distributions  
212 along the dendritic tree are similar between PV- and SST-INs remains unknown. We thus  
213 investigated the synaptic distributions along the dendritic tree of PV and SST-INs in L2/3 of S1  
214 (**Fig. 3A**). To estimate the density of glutamatergic synapses we used transgenic mice in which  
215 PSD95 (a protein involved in the structure of glutamatergic synapses) was conditionally tagged  
216 with a fluorescent marker, mVENUS (42, 43). PSD95 puncta along different dendritic locations  
217 in both PV- and SST-INs were imaged using immunohistochemistry and Confocal/STED  
218 microscopy. To probe if synapse density was uniform or variable across the dendritic tree, we  
219 compared PSD95 puncta density at proximal ( $< 40 \mu\text{m}$  from soma) and distal ( $\sim 100 \mu\text{m}$  from the  
220 soma) dendritic regions. We found that the densities of glutamatergic synapses greatly varied  
221 between proximal and distal locations in PV-INs. The proximal locations exhibited a high density  
222 of synapses while the distal locations had a much lower density along individual dendritic branches  
223 (**Fig. 3B,C**). On the other hand, the density of synapses did not seem to vary along the dendritic  
224 tree in SST-INs (**Fig. 3D-F**).

225 We further investigated such differences in synapse distribution using a publicly available  
226 electron microscopy dataset corresponding to the full reconstruction of a  $1 \text{ mm}^3$  sample of the  
227 mouse visual cortex (44). We combined their databases of (1) proof-read interneuron  
228 identifications (45), (2) 3D reconstructions of single cell morphologies (44) and (3) cell-to-cell  
229 synapse identifications (46) to estimate synaptic distributions along the dendritic tree of INs with  
230 great spatial resolution (**Fig. 3G,H**, see Methods). In this dataset, inhibitory neurons (INs) are  
231 morphologically classified, allowing us to focus on basket cells and Martinotti cells as  
232 representative proxies for parvalbumin-expressing (PV) and somatostatin-expressing (SST) INs,  
233 respectively (2, 47). Consistent with our PSD95 quantifications, the density of synaptic contacts  
234 in basket cells gradually decreases with increasing distance from the soma. The synaptic  
235 distribution peaks in the very proximal regions, exhibiting a strongly negative slope and highly  
236 skewed distributions, **Fig. 3H-J**). On the other hand, the distribution of synapses along the  
237 dendritic tree of Martinotti cells was relatively constant along dendritic branches, with distal  
238 regions displaying similar densities as compared to proximal regions (medial peak location,  
239 negligible slope and weakly-skewed distributions, (**Fig. 3H-J**). Therefore, the major functional

240 dendritic integration properties in PV- and SST-INs described in Figures 1-2 are associated with  
241 remarkable distinct synapse distributions, suggesting two markedly different morpho-functional  
242 organization principles in these two prominent IN subtypes.

243

244 *Two different strategies to enhance EPSP-spike coupling in thin dendritic structures.*

245

246 We next used computational modelling to examine how the two specific dendritic  
247 strategies (proximally-biased synapse distributions + low synaptic NMDAR content vs. uniform  
248 synapse distribution + higher density of synaptic NMDAR) influence integration of synaptic  
249 inputs. We hypothesized that integration properties and synaptic distributions can generate unique  
250 functional features of PV- and SST-INs.

251 For this purpose, we built detailed biophysical models of PV- and SST-INs based on the  
252 morphological reconstructions of basket and Martinotti cells respectively, and their own synaptic  
253 locations from EM data shown in **Fig. 3G,H**. Synaptic and biophysical properties were constrained  
254 by experimental measures (**Table S1-3, Methods**). For each dendritic integration strategy, we  
255 looked for an alternative “null” hypothesis. In the case of SST INs, this was naturally provided by  
256 the removal of the NMDAR component in synaptic transmission. For the PV-IN integration  
257 strategy, we generated a uniform surrogate of its synaptic distribution by redistributing synapses  
258 on each branch according to a constant linear density. This operation simulated a uniform  
259 distribution of glutamatergic synaptic inputs onto dendrites of PV INs. We found that only PV-  
260 INs exhibited significant deviations between the real distribution and the uniform surrogate (**Fig.**  
261 **4A, Fig. S8**).

262 In our glutamate uncaging experiments, we observed sublinear integration in PV-INs,  
263 while sublinear integration in SST-INs was revealed only upon blocking NMDARs. Based on  
264 these findings, we hypothesized that proximally-biased synaptic distribution or the presence of  
265 NMDARs might compensate for sublinear integration, as predicted by cable theory (33, 48). This  
266 would increase synaptic efficacy and ultimately enhance EPSP-to-spike coupling. To test this, we  
267 generated input patterns by randomly picking a sparse subset of synapses (4%, see **Fig. S9**) located  
268 on a distal (160-200  $\mu\text{m}$ ) and proximal (20-60  $\mu\text{m}$ ) segment of a single dendritic branch (**Fig. 4A**)  
269 and simulated the arrival of spatially clustered synaptic inputs that are known to engage sublinear  
270 integration. This resulted in a small number of synapses (from 2 to 8) that we activated  
271 independently and quasi-synchronously (0.5ms delay) to compute the “linear” and “observed”  
272 responses in each of the segments (**Fig. 4B**).

273 For PV-INs, the surrogate, uniform synapse distribution exhibited the distance-dependent  
274 sublinear behavior expected for dendrites acting mostly as passive cables (**Fig. 4B**, grey). In the  
275 “real” scenario with proximally biased synapse distribution, however, the decreased density of  
276 distal synapses resulted in fewer synapses within the 4% cluster (see  $n_{\text{syn}}=2$  vs  $n_{\text{syn}}=5$  in the  
277 “uniform” surrogate, **Fig. 4A**), preventing synaptic saturation. This is evidenced by a significant  
278 reduction in sublinear summation in distal segments (**Fig. 4B-Ci**). On the other hand, the increased  
279 number of synapses in proximal segments (in the “real” distribution case compared to the  
280 “uniform” surrogate) did not strongly increase sublinearity (**Fig. 4Ci**). Therefore, assuming PV-  
281 INs receive signals that are randomly distributed over the dendritic branch, proximally-biased  
282 synapse distributions can reduce sublinear dendritic integration in distal dendritic segments and  
283 limit signal loss by reducing the size of active synaptic clusters (**Fig. 4Di**).

284 As expected, in the case of SST-INs, the “no-NMDA” surrogate also exhibited a distance-  
285 dependent sublinear behavior with strong distal suppression and near-linear integration in proximal



286 dendritic segments (**Fig. 4Bii**, purple). Activation of NMDARs completely abolished this effect  
287 during multi-input integration (**Fig. 4Cii**) due to the involvement of the slow NMDAR excitatory  
288 conductance (**Fig. 4Bii**, orange). Furthermore, in agreement with our glutamate uncaging results,  
289 increasing the number of active synapses during multi-input integration (especially in distal  
290 dendritic segments) resulted in supralinear integration (**Fig. S9**). We conclude that the presence of  
291 a strong NMDAR component observed in the dendritic program of SST-INs not only limits  
292 sublinear integration, but it also amplifies coincident synaptic activity along dendrites, especially  
293 in distal dendritic locations, like PNs (**Fig. 4Dii**).

294 Finally, we analyzed the impact of the differential anatomical and functional synaptic  
295 strategies exhibited by PV and SST cells on firing dynamics under a more realistic setting.  
296 Specifically, we tested the ability of synaptic stimulation to elicit action potentials on a *per branch*  
297 basis. Neuronal activity was simulated in response to increasing synapse number  $n_{syn}$  (randomly  
298 picked on the branch), on top of a stationary excitatory and inhibitory background to mimic *in*  
299 *vivo*-like activity (**Fig. 4E**, see **Methods**). PV-INs with real synaptic distributions were  
300 substantially more effective in driving spiking activity than with uniform distributions (**Fig.**  
301 **4F,Gi**). Likewise, SST-INs exhibited a substantial decrease in the spiking probably in response to  
302 synapses devoid of NMDARs (**Fig. 4F,Gi**, despite a boost of the AMPAR conductance weight to  
303 match EPSP size at -60 mV, see **Methods**).

304 We conclude that the differential morpho-functional dendritic integration strategies  
305 exhibited by L2/3 PV- and SST-INs represent two distinct strategies to increase synaptic efficacy.  
306 Synapses on PV-INs are positioned strategically to reduce the likelihood of sublinear integration,  
307 especially in distant dendritic areas. Conversely, SST-INs take advantage of the NMDARs to  
308 enhance coincident dendritic activity all along their dendritic arbor.

309  
310 *Different dendritic integration strategies in PV- and SST -INs result in temporally-distinct output*  
311 *firing dynamics.*

312  
313 While both dendritic integration mechanisms can compensate for the synaptic strength  
314 shortcomings of sublinear summation, the slow NMDA receptor conductance is likely to produce  
315 a very different temporal profile of IN spiking (49). Indeed, in our detailed biophysical models, a  
316 striking difference between PV- and SST-INs was their specific temporal window for input  
317 selectivity and output spike patterns (**Fig. 5A**). The spiking pattern of the PV-IN model accurately  
318 transmitted the amplitude and width of step stimulations (**Fig. 5A**, red, note the similarity between  
319 input steps and output rate responses) while the SST-IN model would produce a more integrative  
320 and long-lasting IN-spiking response due presence of the slow NMDAR-mediated conductance  
321 (**Fig 5A**, orange, note the equal rate levels in the second step despite a twice weaker amplitude,  
322 note also the delayed responses outside the stimulus window). To generate testable predictions,  
323 we simulated a more realistic input pattern by stimulating the IN models with temporally correlated  
324 excitatory and inhibitory activity (**Fig. 5B**, the input strength was scaled so that average output  
325 firing lies in the 10-15 Hz range, see **Methods**). We then analyzed the temporal dynamics of the  
326 two IN models by computing the cross-correlation function of its output spiking rate (after trial  
327 averaging) with the input signal (in grey, in **Fig. 5A**). The fast and linear report of response  
328 duration of PV-IN resulted in a sharp inhibition closely following the input temporal correlations  
329 (**Fig. 5C**, captured by a sharp cross-correlation half-width in **Fig. 5D**). The broader and long-  
330 lasting responses of SST INs resulted in a delayed and extended firing dynamics (**Fig. 5C**, captured

331 by a broader cross-correlation half-width in **Fig. 5D**). These initial predictions are consistent with  
332 the known coincidence detection mode of PV-INs and the more integrative mode of SST-INs (16).

333 We tested the above predictions in a publicly available dataset of single unit recordings  
334 from the mouse visual cortex in response to natural movies presentation, in which PV- and SST-  
335 IN firing was identified by photo-tagging (50) (**Fig. 5E**, see **Methods**). Specifically, we computed  
336 the time-varying firing rate of each IN type by pooling the positive units of a single session  
337 ( $n=24\pm 14$  per session,  $N=9$  sessions for PV-INs,  $n=8\pm 6$  per session,  $N=12$  sessions for SST-INs).  
338 Because both PV- and SST-INs are primarily driven by local cortical excitation (51), we estimated  
339 the input signal by computing the time-varying rate of 100 randomly picked, non-photo-tagged  
340 (negative) units in the recordings, assumed to originate from putative PNs (**Fig. 5F**). We next  
341 computed the cross-correlation between input (from negative units) and IN rates (from positive  
342 units) (**Fig 5G**). In accordance with our model predictions, we observed a wider temporal window  
343 in SST INs in the cross-correlation function, as compared to PV INs (**Fig. 5G**). This observation  
344 was highly consistent across sessions and movies, as measured by the half-width of the cross-  
345 correlation function (**Fig. 5H**). We conclude that the population dynamics of PV- and SST-INs  
346 have temporal features compatible with our observed distinct dendritic integration strategies.

347 Importantly, PV- and SST-INs do not vary only in their dendritic integration strategies, and  
348 therefore other mechanisms could underlie these differences. For example, synaptic facilitation at  
349 the excitatory to SST-INs synapses could also contribute to such extended temporal features. To  
350 specifically address this concern, we tested a more specific prediction of the model: the wider and  
351 delayed responses of SST-INs should be significantly reduced upon removal of NMDARs from  
352 this IN subclass (**Fig. 5C**). To this purpose, we conditionally knocked out NMDARs by crossing  
353  $\text{GluN1}^{\text{fl/fl}}$  with SST-Cre mice (SST:GluN1-KO mice). Importantly, despite lacking NMDARs,  
354 glutamatergic synaptic transmission and short-term plasticity at L2/3 PNs SST-INs synapses were  
355 not altered in SST:GluN1-KO mice (**Fig. S10**). We then expressed the genetically encoded  $\text{Ca}^{2+}$   
356 sensor GCaMP6s in SST INs and recorded their activity in V1 of both control mice and mice  
357 lacking NMDARs specifically in SST-INs (**Fig 5I** and **Methods**). Fluorescence signals were  
358 deconvolved to remove the calcium-sensor dynamics (see **Methods**). Awake mice were presented  
359 full-field grating stimuli at two contrasts (0.5 and 1) and 8 orientations. We analyzed the properties  
360 of the stimulus-evoked activity (**Fig. 5I**). In control conditions, SST-INs responded robustly to  
361 visual stimuli (**Fig. 5J**). We observed a significant decrease of SST-INs responsiveness at half and  
362 full contrast in SST:GluN1-KO mice, thus supporting the role of the NMDARs in shaping SST-  
363 INs activity (**Fig. 5J**). In agreement with the results of **Fig. 5A-H**, visually evoked responses of  
364 SST INs exhibited much sharper temporal dynamics in SST:GluN1-KO mice as compared to their  
365 WT littermates (**Fig. 5K**). Our findings indicate that the SST-IN-specific dendritic strategy to  
366 integrate glutamatergic synapses using NMDARs plays an important role in shaping the temporal  
367 features of SST-mediated firing behavior in response to visual stimuli.

## 368 369 **Discussion**

370  
371 In this study, we provide an in-depth analysis of the dendritic integration properties of two  
372 major inhibitory neuron classes, PV- and SST-INs, which are characterized by distinct  
373 connectivity properties and functional roles within cortical networks (2–4). Despite the extensive  
374 body of evidence examining dendritic integration in cortical PNs, little is known about how  
375 synaptic activity is processed by dendrites of cortical INs. We found that PV- and SST-INs use  
376 two different strategies to integrate inputs along their dendritic trees. Interestingly, our model

377 simulations predict that variations in the density of synaptic NMDARs is a major contributor for  
378 such differential dendritic integration properties. Previous studies reported that the density of  
379 NMDARs is quite variable across IN classes (49, 52), but the functional relevance of such different  
380 expression levels of NMDARs was unclear. We propose that differences in the content of synaptic  
381 NMDARs across populations of interneurons contribute to generate functional diversity in their  
382 dendritic operations and ultimately define their role during *in vivo* network activity. Such a  
383 hypothesis is also supported by recent findings in PNs where variability of NMDA-to-AMPA ratio  
384 was reported to result in the presence of different modes of dendritic integration in L5b PNs of the  
385 retrosplenial cortex to for distinct long-range inputs (53).

386 The present results suggest that, in addition to the known morphological, transcriptomic,  
387 and electrophysiological differences, dendritic integration properties vary across the distinct  
388 populations of cortical interneurons. Numerical simulations highlight that such a variability in  
389 integration properties plays a crucial role in shaping the distinctive functional characteristics of  
390 specific interneuron classes, particularly PV- and SST-INs. In PV-INs, dendrites behave mostly as  
391 passive cables, due to the combination of reduced NMDAR levels, and low input resistance. This  
392 leads to a synaptic integration mechanism that enables rapid membrane voltage deflections in  
393 response to increased excitatory synaptic activity. Such a dendritic performance allows precise  
394 temporal response of PV-INs to variations in pyramidal cell activity. These properties seem to be  
395 particularly well tailored for PV-INs to fulfill their important role as sharp coincidence detectors,  
396 to sharpen sensory inputs through powerful and precise feedforward inhibitory networks, and drive  
397 fast cortical brain oscillations (18). Conversely, in SST-INs, the presence of slow NMDARs along  
398 dendrites extends the window of temporal integration, the efficacy of synaptic activity in driving  
399 SST-INs firing. This extended temporal window appears important for providing feedback  
400 inhibition to cortical microcircuits using glutamatergic synapses that are known to be particularly  
401 inefficient because of low release probability (19, 20).

402 Surprisingly, we found that the different dendritic integration modes of PV and SST INs  
403 were associated with different distributions of glutamatergic inputs along their dendritic trees.  
404 Ultimately, the engagement of dendritic operations relies on the spatial and temporal patterns of  
405 activation of many synaptic inputs. Currently, information about the patterns of synaptic activity  
406 reaching dendrites of PV- and SST-INs *in vivo* is lacking. Yet, our examination of the distribution  
407 of synapses reveals a non-uniform pattern along the dendritic branches of PV-INs. This raises the  
408 hypothesis that, during cortical processing, the number of local simultaneously active synapses  
409 (active clusters) along PV-IN dendrites may be variable, which could have an impact on non-linear  
410 dendritic engagement. In the cerebellum, gap junctions have been reported to compensate for  
411 sublinear integration (14). Here, we propose that gradients of synapse distributions in PV INs may  
412 have a similar effect by reducing the number of coactive synapses in distal dendritic regions, where  
413 input resistance is particularly elevated, and dendritic saturation is easily achieved. Interestingly,  
414 in dendrites of SST-INs where synapses contain higher density of NMDARs, this effect was much  
415 less pronounced. Altogether, our data suggest a general scenario in which INs use a variety of  
416 dendritic integration strategies to enhance the efficiency of synaptic transmission into post-  
417 synaptic spikes (54), but tailor the timing of that activity to the specific functional attributes of  
418 each IN population.

419 The asymmetric distribution of synapses that we report here does not preclude the  
420 occurrence of sublinear integration in the dendrites of PV-INs. Ultimately, the clustered activation  
421 of inputs depends on the spatial and temporal arrival of synaptic activity in dendrites *in vivo*,  
422 determined not only by the hardwiring inputs but also by their timing of activation. Non-linear

423 dendritic operations, particularly in PV-INs in the hippocampus, have been proposed to be  
424 important for enhancing memory encoding and to detect network ripple activity in PV-INs (9–12,  
425 55). However, such diverse functions of PV-INs require precise hardwiring and spatio-temporal  
426 organization of inputs into PV-INs to contribute to the reported physiological phenomena. Here,  
427 we propose that, in a more general framework without assuming a specific connectivity motif, PV-  
428 INs bias their input distribution to proximal regions of the soma to maximize EPSP-to-spike  
429 coupling and reduce saturation in the distal parts of their dendrites. Nevertheless, future studies  
430 are crucial to dissect the physiological patterns of synaptic activity that occur in PV- as well as in  
431 SST-INs. Additionally, in our study, we primarily focused on studying the integration of voltage  
432 signals. It is known that calcium signaling might not necessarily follow the same integration path  
433 as the synaptically-evolved EPSP (6).

434 Overall, we describe two simple yet clever strategies employed by PV- and SST-INs to  
435 prevent synaptic saturation at their distal dendrites: removal of NMDARs and concentration of  
436 glutamatergic synapses towards the soma in PV-INs, and enrichment of NMDARs all along the  
437 dendrites in SST-INs. These differential strategies result in profoundly different transformations  
438 of excitatory recruitment signals into firing activity by each IN subclass, ultimately shaping their  
439 distinct roles within cortical networks.

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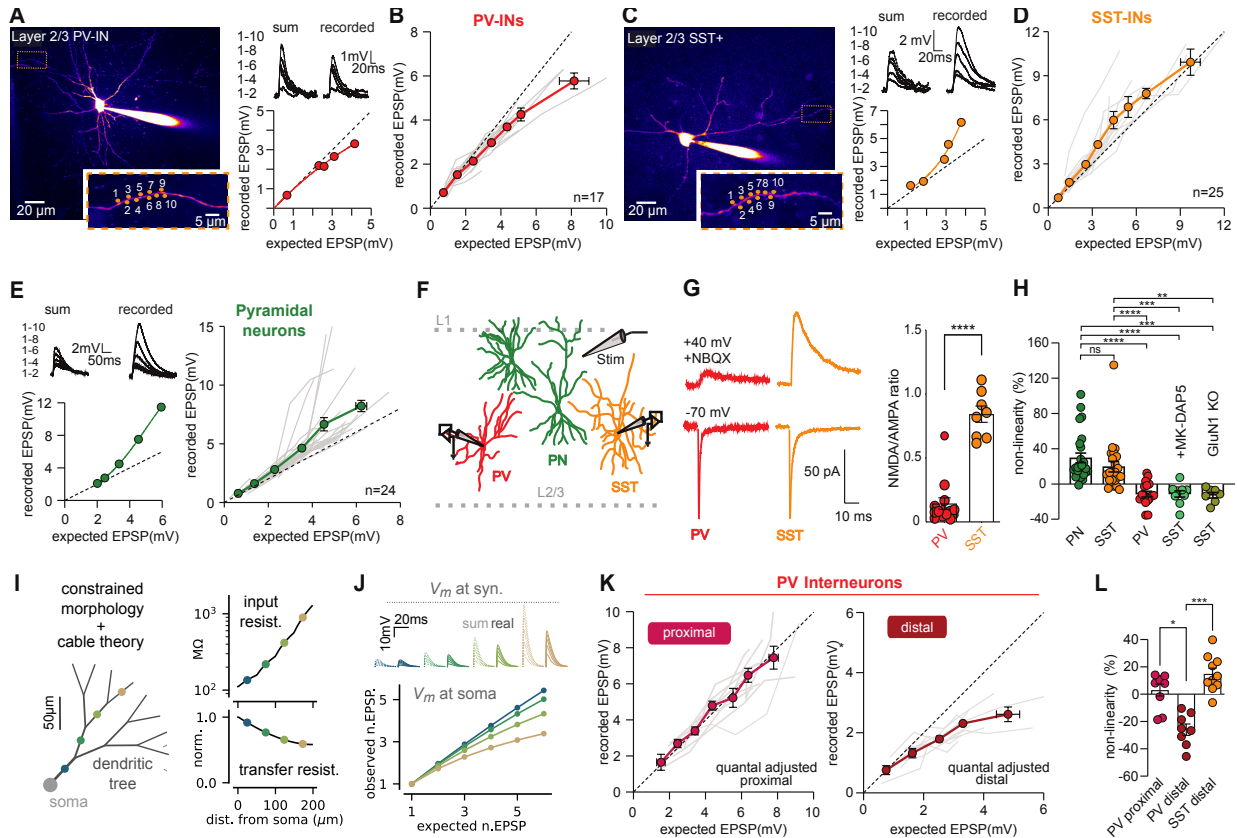


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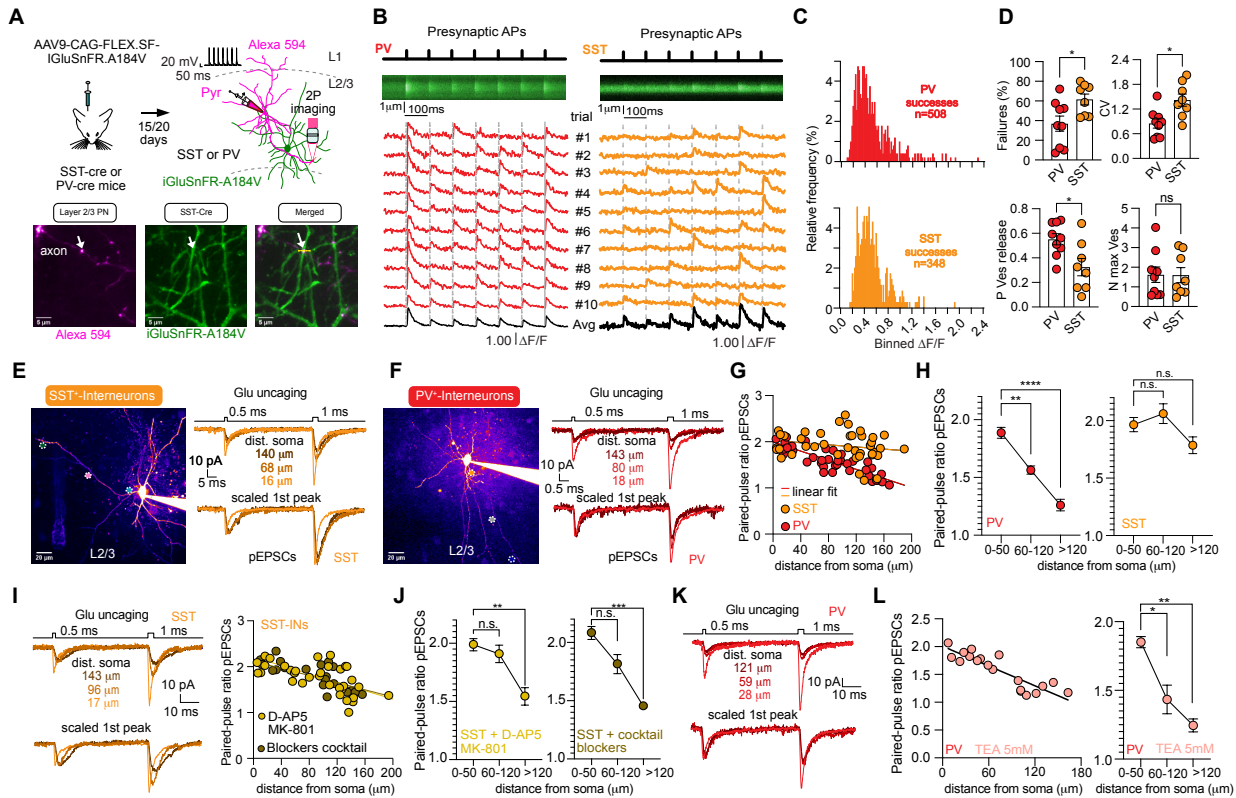
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698 **Figure 1: PV- and SST-INs display different dendritic integration properties.** **A)** (left) Two-  
 699 photon laser scanning microscopy (2PLSM) image (maximum-intensity projection, MIP) of a PV-  
 700 IN patch loaded with Alexa-594 (10  $\mu$ M). The inset shows a selected dendrite and uncaging sites  
 701 for 10 synapses. (right) Example of photolysis-evoked EPSPs in a dendrite with increasing  
 702 number of stimulated synapses (from locations in A, activated at 0.12 ms intervals). The traces compare  
 703 the calculated linear sum of individual responses to the recorded response from quasi-synchronous  
 704 synapse activation. The input-output curve shows recorded somatic EPSP amplitudes versus the  
 705 expected linear sum. **B)** Summary plot of input-output curves for dendrites of PV-INs. Single grey  
 706 lines represent individual experiments (n=17) and in red the binned average. **C-D)** Same as A-B,  
 707 but for SST-INs. **E)** Same as A-B, but for pyramidal neurons. **F)** Schematic representation of  
 708 experimental conditions used to stimulate local excitatory connections and record glutamatergic  
 709 synaptic activity in PV- and SST-INs in layer 2/3 of primary somatosensory cortex. **G)**  
 710 Representative traces (left) and summary plot (right) of EPSCs recorded at -70 mV and +40 mV  
 711 in PV- and SST-INs (n=8; p<0.001, Mann-Whitney). **H)** Percentage of non-linearity obtained in  
 712 PYR (green, n=25), SST-INs (orange, n=23), PV-INs (red, n=17), SST INs in the presence of

713 NMDA blockers (light green, n=14) and SST-INs from GluN1-KO mice (khaki green, n=7). The  
714 data are presented as average  $\pm$  SEM; \*\*p<0.001, \*\*\*p<0.0001, \*\*\*\*p<0.00001, Kruskal-Wallis  
715 test with Dunn's correction. **I**) Properties of the simplified model of PV-IN integration. (*left*)  
716 Schematic of the morphology with a somatic compartment and symmetric-branching dendritic  
717 tree. (*right*) Input resistance and transfer resistance profiles along the dendritic tree relative to soma  
718 distance. **J**) Input-output curve in the simplified model. (*top inset*) Depolarization values from  
719 quasi-synchronous input of increasing number of synapses (1 to 6 synapses) at various dendritic  
720 locations (color-coded as in I). Dashed and solid lines show linear prediction (sum) and observed  
721 (real) responses. (bottom) Relationship between expected (sum) and observed (real) peak EPSP  
722 values at different dendritic locations (color-coded), normalized to single event EPSP values. **K**)  
723 Subthreshold input-output relationship of pEPSPs in proximal (<40  $\mu$ m from soma) and distal (100  
724  $\mu$ m from soma) dendrites using quantal adjusted pEPSP amplitude. Dashed line indicates slope of  
725 1. **L**) Summary of non-linearity percentage in proximal (cardinal red) and distal (brick red) PV-  
726 INs and distal SST-INs dendrites (orange) using quantal adjusted EPSP amplitude. Data are  
727 average  $\pm$  SEM, \*p<0.01, \*\*\*p<0.0001, Kruskal-Wallis test with Dunn's correction  
728



730

731 **Figure 2: Multivesicular release summates sublinearly in PV-INs dendrites and**

732 **predominantly linearly in SST-INs dendrites. A)** (Top) Methodology to study neurotransmitter

733 release at synaptic contacts between PN and PV- or SST-INs using iGluSNFR-A184V expressed

734 in PV- or SST-INs. Glutamate release was evoked from L2/3 PN axons via current injection. (Bottom)

735 Two-photon images show a patch-loaded PN axon (red) and SST-INs expressing iGluSNFR-

736 A184V (green). **B)** Averaged line scan (10 images) of iGluSNFR-A184V fluorescence (green) at

737 contact points between L2/3 PN boutons and PV/SST-IN dendrites after AP (7 APs @ 10 Hz)

738 initiation. Time series traces (10) show mean fluorescence over 1 μm (white line), with the black

739 trace as the average (ΔF/F). **C)** Histogram of ΔF/F amplitude distributions for PV (red) and SST-

740 INs (orange) iGluSNFR events. **D)** Summary of failure rates and coefficient of variation for L2/3

741 PN synaptic contacts in SST- (n=8) and PV-INs (n=9). Data include release probability (Pves) and

742 maximum vesicles released per AP (N max Ves), (\*p<0.01, Mann-Whitney test). **E)** Two-photon

743 image of an SST-IN loaded with Alexa 594, with glutamate uncaging at three dendritic locations.

744 Somatically recorded EPSCs from photolysis at two pulse durations. **F)** Similar to **E** for PV-INs.

745 **G)** Summary of paired-pulse ratios of photolysis-evoked EPSCs for PV and SST-INs. **H)** Paired-

746 pulse ratios binned by distance from soma for PV and SST-INs, (\*\*p<0.001, p<0.00001, Kruskal

747 Wallis test with Dunn's correction). **I, J)** Similar analysis in the presence of NMDA receptor

748 antagonists, alone (**I**) or with NMDA and VGCC blockers (**J**), (\*\*p<0.001, p<0.00001, Kruskal

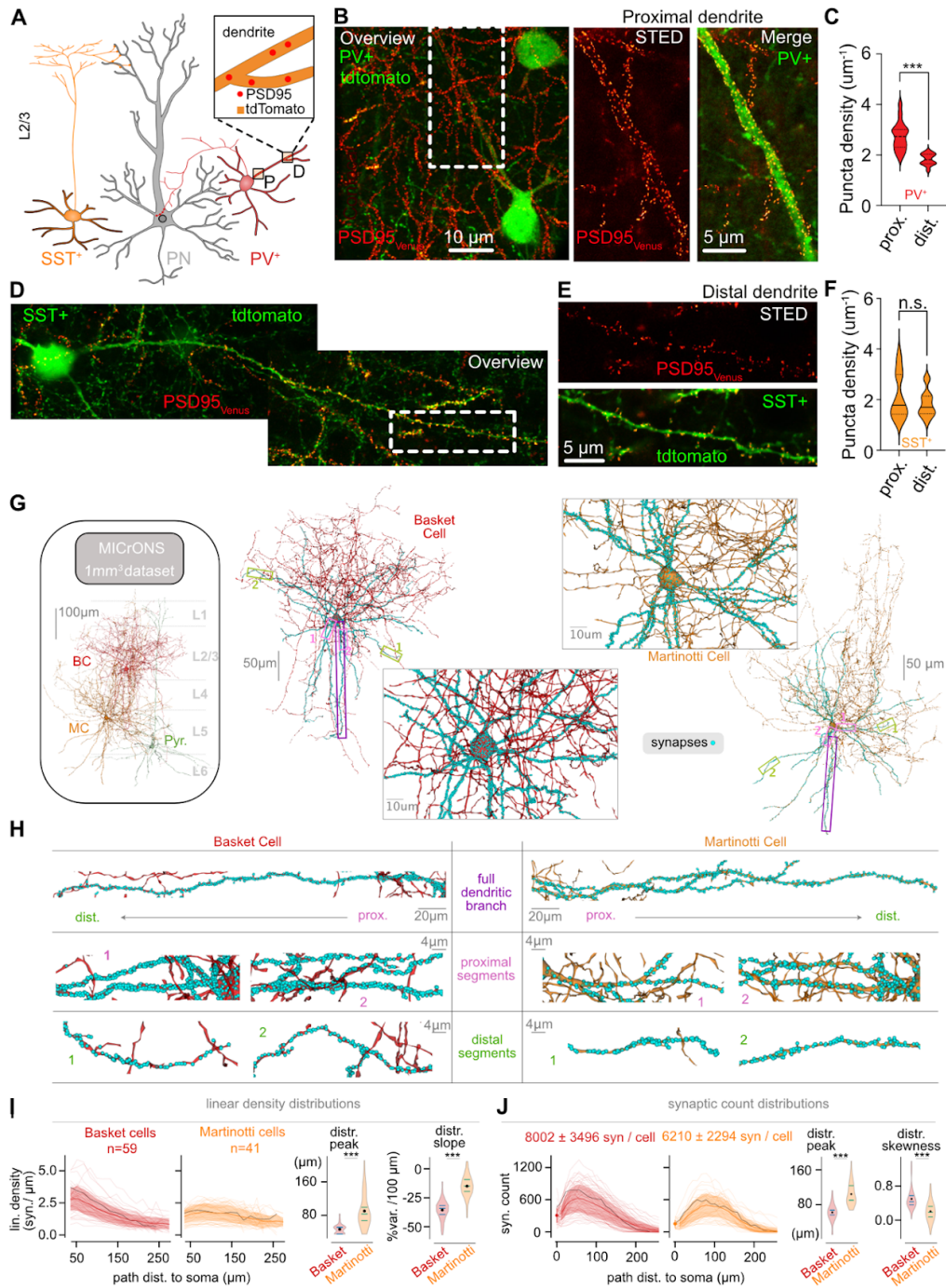
749 Wallis test with Dunn's correction). **K, L)** Similar analysis for PV-INs with TEA (5 mM),

750 (\*\*p<0.001, p<0.00001, Kruskal Wallis test with Dunn's correction). Data are mean ± SEM.

751

752

Figure 3



754

755 **Figure 3: Distinct synaptic distributions in the dendritic trees of Basket and Martinotti INs.**

756 **A)** Illustration of experimental approach used to quantify glutamate synapse distribution along

757 dendrites of SST and PV-INs. PSD-95 proteins were selectively labelled in PV- and SST-INs and

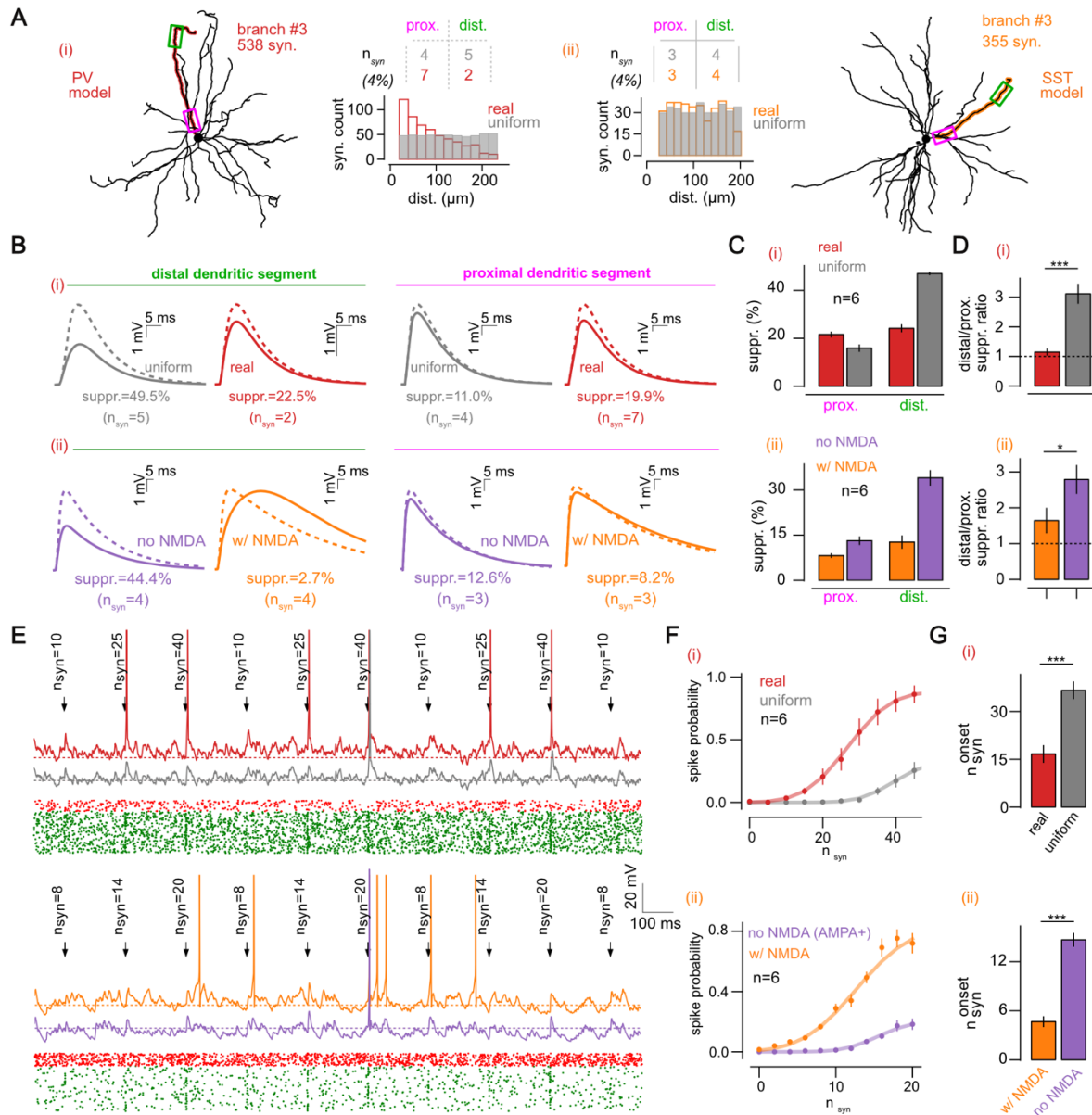
758 quantified at two different distances along individual dendritic branches. **B)** (left) Overview image

759 of PV-INs with proximal dendrites labelled with tdTomato (green) and PSD95<sub>Venus</sub> (red). (Middle) STED image of dendritic location defined on the left. (right) Merged between tdTomato

760

761 obtained in confocal mode (green) and PSD95venus (red) labelling obtained in STED. **D, E**) Same  
762 as **B** but for SST-INs. Note the absence of PSD-95 puncta close to soma but clear labelling in distal  
763 dendrites. **C, F**) Summary plot of density of PSD-95 puncta in proximal (less than 40  $\mu\text{m}$ ) and  
764 distal (approximately 100  $\mu\text{m}$ ) dendritic location in PV- and SST-IN dendrites ( $PV_{\text{proximal}}$ ,  $n=33$ ;  
765  $PV_{\text{distal}}$ ,  $n=12$ ,  $p<0.0001$ , Mann-Witney test;  $SST_{\text{proximal}}$ ,  $n=21$ ;  $SST_{\text{distal}}$ ,  $n=14$ ,  $p>0.01$ , Mann-  
766 Witney test). **G**) Example of basket and Martinotti INs reconstructions from the millimeter-scale  
767 volumetric electron microscopy MICrONS dataset (48). Blue dots indicate the location of  
768 identified synapses along the dendrites of a basket and a Martinotti Cell. **H**) Illustration of synapse  
769 distributions in individual dendritic branches from reconstructed Basket (*left*) and SST-INs (*right*)  
770 displayed in **G**. Images display synapses along full individual dendritic branches as well at  
771 proximal and distal dendritic segments. **I**) Linear synapse density distributions (*left*, see Methods)  
772 for all basket ( $n=59$ ) and Martinotti ( $n=41$ ) cells as a function of the distance from soma. (*right*)  
773 Violin plots of distribution peaks (Basket vs Martinotti,  $p=1\text{e-}11$ , Mann-Whitney test) and  
774 distribution slopes as a variation per 100 $\mu\text{m}$  (Basket vs Martinotti,  $p=6\text{e-}14$ , Mann-Whitney test)  
775 for the plots on the left. **J**) Synaptic count (*left*, see Methods) for all basket ( $n=59$ ) and Martinotti  
776 ( $n=41$ ) cells as a function of the distance from soma. (*right*) Violin plots of distribution peaks  
777 (basket vs Martinotti,  $p=2\text{e-}13$ , Mann-Whitney test) and distribution skewness (basket vs  
778 Martinotti,  $p=1\text{e-}8$ , Mann-Whitney test) for the plots on the left.

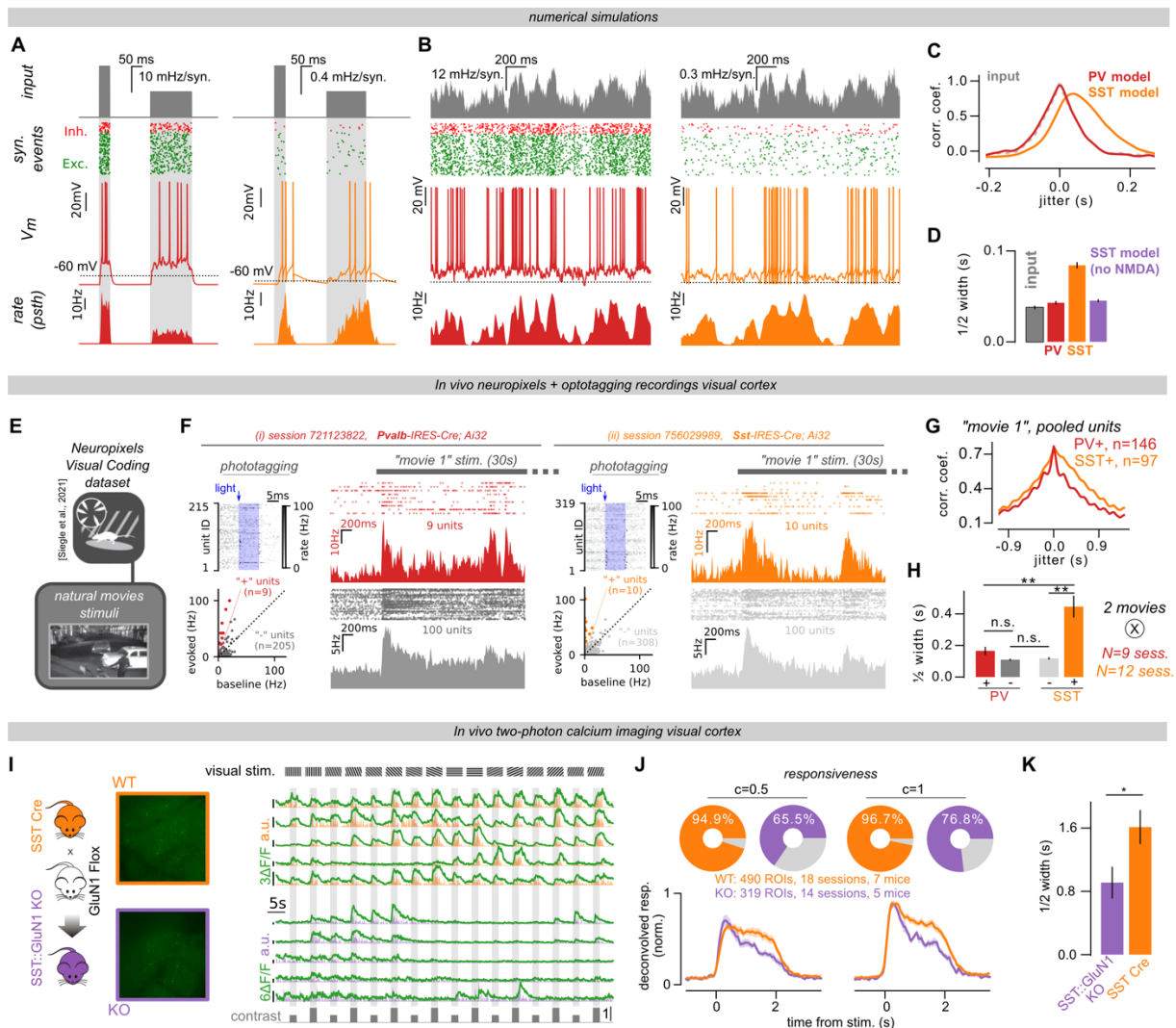




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781 **Figure 4: The PV- and SST-IN dendritic programs improve distal signal transmission and**  
 782 **optimize synaptic efficacy through different mechanisms.** **A)** Morphology of the EM  
 783 reconstructed basket cell (i) and Martinotti cell (ii) used as PV- and SST-IN models, respectively.  
 784 In the insets, we show the real distributions (colored bars) of synapses on a single representative  
 785 branch together with the uniform surrogate distribution (plain gray bars). The inset table shows  
 786 the sparse subset of synapses in the distal (green) and proximal (pink) segments. **B)** Example  
 787 numerical simulations of  $V_m$  response following the quasi-synchronous stimulation of the synapses  
 788 in the distal (green, left) and proximal (pink, right) segments in the PV-IN (i) and SST-IN (ii)  
 789 models. We show the observed response (plain line) and the expected response (dashed line) from  
 790 the individual event responses. We compare each model to its control situation (i, grey, uniform  
 791 distribution), (ii, purple, no-NMDA). **C)** Suppression of EPSP amplitude between observed

792 response and linear predictions (see annotations in **B**) in the PV-IN (i, red) and SST-IN (ii, orange)  
793 models with their own control in the distal (green) and proximal (pink) segments. **D**) Ratio of  
794 suppression between the distal and proximal segments in the PV-IN model (i, red, with its “uniform  
795 distribution” control in grey,  $n=6$  branches,  $p=3e-2$ , Wilcoxon test) and SST-IN model (ii, orange,  
796 with its “no NMDA” control in purple,  $n=6$  branches,  $p=3e-2$ , Wilcoxon test). **E**)  $V_m$  dynamics at  
797 the soma following background and stimulus-evoked activity in the dendritic branch shown in **A**.  
798 Synaptic stimulation consists in the quasi-synchronous activation of increasing number of  
799 synapses  $n_{syn}$  (see annotations). We show the PV-IN model (red, with its “uniform distribution”  
800 control in grey) and SST-IN model (orange, with its “no NMDA” control in purple). **F**) Summary  
801 plot of the spike probability (in a 50ms post-stim. window) as a function of the number of synapses  
802  $n_{syn}$  in the stimulus. **G**) Onset response level  $n_{syn}^{onset}$  in the PV-IN model (red, with its “uniform  
803 distribution” control in grey,  $p=3e-2$ ,  $n=6$  branches, Wilcoxon test) and SST-IN model (orange,  
804 with its “no NMDA (AMPA+)” control in purple,  $p=3e-2$ ,  $n=6$  branches, Wilcoxon test). Onset  
805 level  $n_{syn}^{onset}$  is defined as the input level where the “Erf” fit goes above spike-proba=0.05. Results  
806 are shown as mean  $\pm$  s.e.m over  $n=6$  branches in panels **C,D,F,G**.  
807



809

810 **Figure 5: The PV- and SST-IN dendritic programs shape two temporally-distinct inhibitory**  
 811 **dynamics in cortical networks.** **A)** Response to packets of excitatory and inhibitory activity  
 812 controlled by current steps in the PV-IN model (red) and in the SST-IN model (orange). We show  
 813 the time-varying rate (top, grey) controlling the generation of excitatory and inhibitory events  
 814 (green and red respectively), a single trial  $V_m$  example (middle) and the time-varying output rate  
 815 built from multiple trials ( $n=24$ ). **B)** Same than **A** but for a time-varying input rate controlled by a  
 816 temporally-correlated stochastic process (see Methods). **C)** Cross-correlation function between  
 817 input rate and PV-IN (red) and SST-IN (orange) rate. We also show the autocorrelation function  
 818 of the input rate (grey). **D)** Positive half-width of the cross-correlation functions (see Methods,  
 819 shown as mean  $\pm$  s.e.m. over  $N=4$  input seeds  $\times$   $n=6$  branches; input vs PV,  $p=6e-3$ ; input vs SST,  
 820  $p=3e-9$ ; input vs SST-noNMDA,  $p=3e-3$ ; PV vs SST,  $p=3e-9$ ; SST vs SST-noNMDA,  $p=3e-9$ ;  
 821 Mann-Whitney test). **E)** Schematic of the Neuropixels dataset from (Siegle et al., 2021). **F)** Single  
 822 session examples in PV-cre and SST-cre (ii) mice. We show the photo-tagging trials (top-left) and

823 the summary analysis to split positive and negative units (see Methods). We show the spiking  
824 events of positive (colored) and negative (grey) units around stimulus onset (right). **G**) Cross-  
825 correlation function between negative unit rates and PV-positive rate (red) or SST-positive rate  
826 (orange) for the natural movie #1 shown in all sessions. **H**) Half-width of the cross-correlation  
827 function in the positive and negative units. PV-cre mice: N=9 sessions x 2 movies, PV+ units vs  
828 PV- units,  $p=7e-2$ , Mann-Whitney test. SST-cre mice: N=12 sessions x 2 movies, SST+ units vs  
829 SST- units,  $p=2e-5$ , Mann-Whitney test. PV- units vs SST- units:  $p=5e-1$ , Mann-Whitney test.  
830 PV+ units vs SST+ units:  $p=3e-3$ , Mann-Whitney test. Results are shown as mean $\pm$ sem over  
831 sessions and movies. **I**) (left) Illustration of the genetic mouse model approach to selectively  
832 remove NMDARs from SST-INs. (Middle) Two-photon image of SST-INs in V1 expressing  
833 GCaMP6s. (Right) Representative fluorescence traces shown as  $\Delta F/F_0$  (green) together with their  
834 deconvolution traces. **J**) Percentage of SST-INs exhibiting a statistically significant positive  
835 response to visual stimuli at both full and half contrast in wild-type subjects and in animals lacking  
836 GluN1 subunits selectively in SST-INs. **K**) Deconvolved responses following stimulus  
837 presentation (average over all orientations) at half and full contrast. In the inset, we show the value  
838 of a Gaussian curve decay in the fitting window highlighted in grey. **L**) Half-width of the evoked  
839 response decay (evaluated by the width parameter of a Gaussian fit in the window highlighted in  
840 grey). SST:WT vs SST:GluN1-KO,  $p=4e-2$ , Mann-Whitney test.

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865 **Author contributions:**

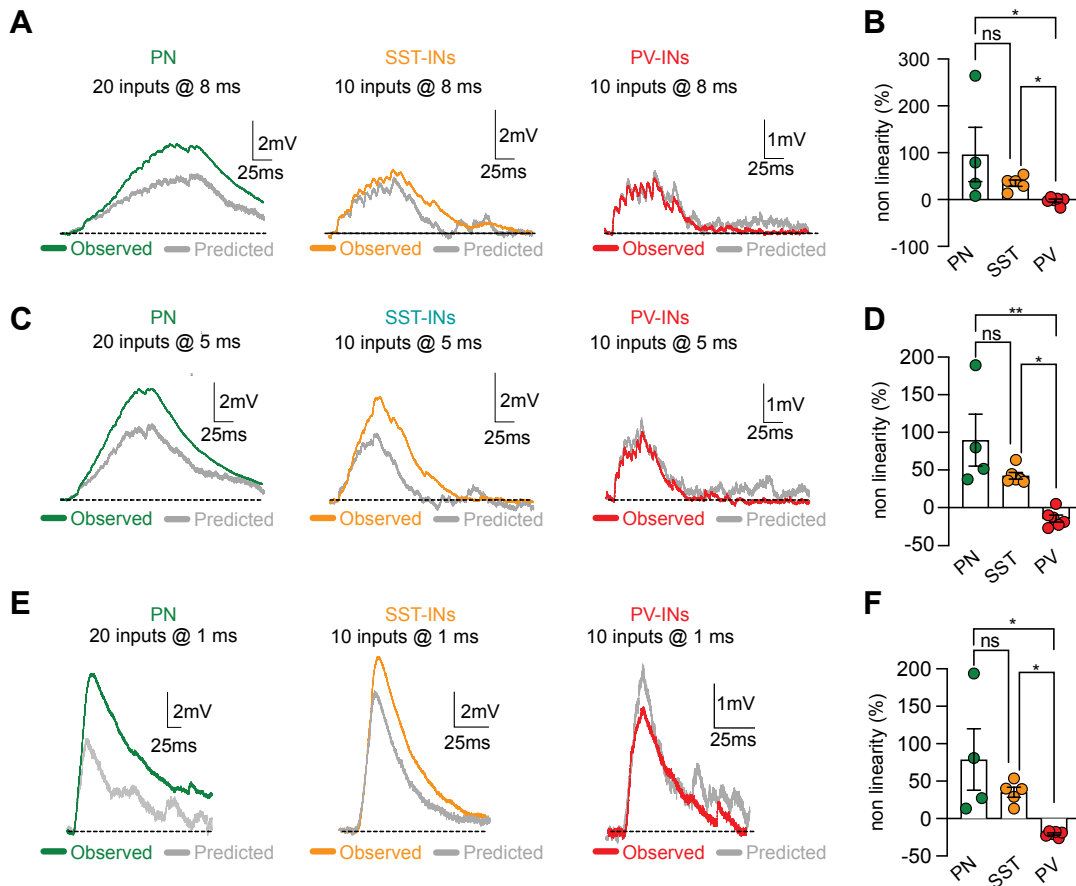
866 Conceptualization: AM,YZ, AB, DD, JL and NR  
867 Methodology: AM,YZ, DhD,EB,AT,GM,LC,PP,AB,DD,JL and NR  
868 Investigation: AM,YZ, DhD,EB,AT,GM, JL and NR  
869 Visualization: AM,YZ, DhD,EB,AT,GM,LC, PP, AB,DD, JL and NR  
870 Funding acquisition: YZ, DD, AB,JL and NR  
871 Project administration: NR  
872 Supervision:AB,DD,JL and NR,  
873 Writing – original draft: AM,YZ,AT,PP,AB,DD,JL and NR  
874 Writing – review & editing: AM,YZ,AT,LC,PP,AB,DD,JL and NR

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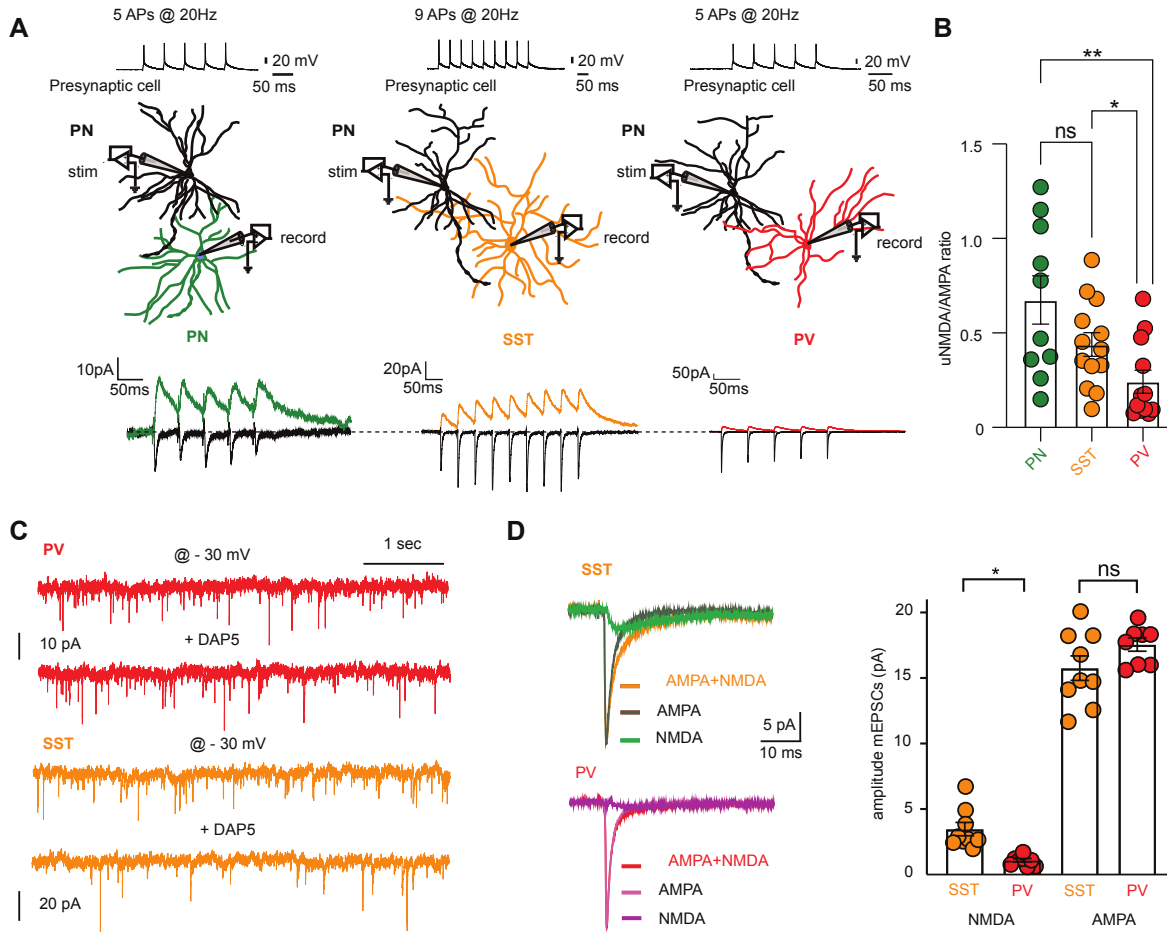
**Competing interests:** Authors declare that they have no competing interests.

878 **Data and materials availability:** The code for the data analysis (EM dataset, Neuropixels  
879 dataset, Imaging dataset) and numerical simulations (simplified morphological model, detailed  
880 biophysical and morphological models) of this study is publicly available at the following link:  
881 <https://github.com/yzerlaut/pv-sst-dendrites>.

882



887 **Figure S1: Dependence of synaptic integration in PN, SST and PV-INs on the time interval**  
 888 **between uncaging events.** **A)** Population average pEPSPs recorded at soma in response to 20  
 889 (PN) or 10 uncaging spots (PV and SST-INs) activated with 8 ms inter pulse interval. Recorded  
 890 average is presented in color while the linear sum is depicted in grey. **B)** Summary plot of non-  
 891 linearity obtained across the different population of cells using 8ms interpulse interval. Data are  
 892 presented as average  $\pm$  SEM with single experiment quantified as dotted color (PN:  $96.36 \pm 57.88\%$ ,  
 893  $n=4$ ; SST:  $+35.08 \pm 6.69\%$ ,  $n=5$ ; PV:  $-2.32 \pm 3.25$ ,  $n=6$ ; PN vs SST  $p > 0.99$ ; PN vs PV  $p = 0.02$ ; SST  
 894 vs PV  $p = 0.03$ ; Kruskal Wallis test with Dunn's correction). **C, D)** Same as A and B, but for 5 ms  
 895 interpulse interval (PN:  $89.66 \pm 34.39$ ,  $n=4$ ; SST:  $42.36 \pm 4.45$ ,  $n=6$ ; PV:  $-14.64 \pm 4.68$ ,  $n=6$ ; PN vs SST  
 896  $p = 0.83$ ; PN vs PV  $p = 0.003$ ; SST vs PV  $p = 0.046$ ; Kruskal Wallis test with Dunn's correction). **E,**  
 897 **F)** Same as A and B, but for 1 ms interpulse interval ( PN:  $78.85 \pm 41.01$ ,  $n=4$ ; SST:  $30.41 \pm 5.83$ ,  
 898  $n=5$ ; PV:  $-20.49 \pm 1.47$ ,  $n=6$ ; PN vs SST  $p > 0.99$ ; PN vs PV  $p = 0.01$ ; SST vs PV  $p = 0.03$ ; Kruskal  
 899 Wallis test with Dunn's correction).

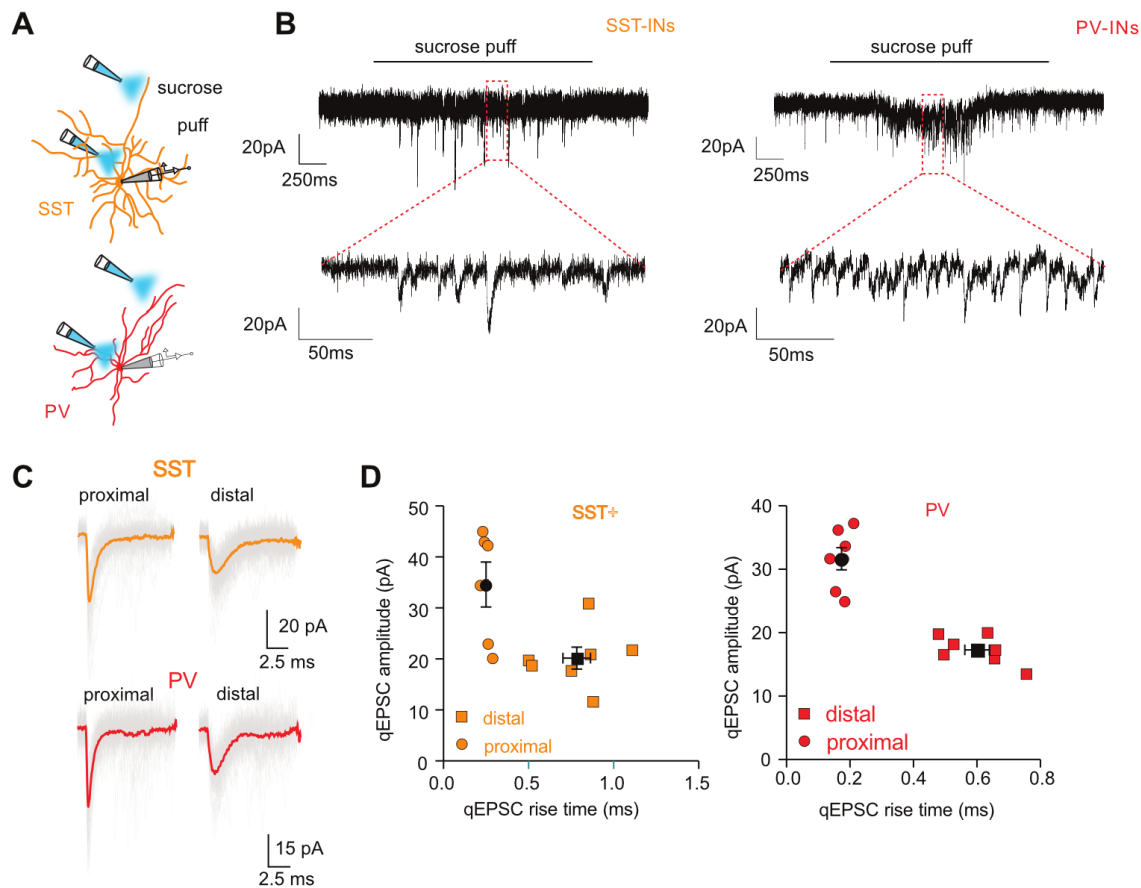


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904 **Figure S2: Different synaptic AMPA-to-NMDA receptor ratio in SST and PV-INs.** **A)** (*top*)  
 905 Schematic representation of the experimental approach used to monitor unitary EPSCs between  
 906 individual L2/3 pyramidal neurons (pyr) and different postsynaptic partners. Unitary AMPA- and  
 907 NMDA-EPSCs were separately recorded by varying the holding membrane potential of the  
 908 postsynaptic potential from -70 mV (uAMPA-EPSCs) to +40 mV (uNMDA-EPSCs). NBQX was  
 909 added to the recording solution at positive potentials to block AMPA+Kainate receptors. Synaptic  
 910 currents were recorded in response to a presynaptic train of action potential evoked at 20 Hz  
 911 through current injection. (*bottom*) Representative AMPA- and NMDA-EPSCs (average of 30  
 912 sweeps) obtained for the different postsynaptic partners. **B)** Summary plots for the experiments  
 913 illustrated in A reported as uNMDA to AMPA ratio (PN n=10; SST n=14; PV n=14). The data are  
 914 reported as mean  $\pm$  SEM (PN:  $0.67 \pm 0.13$ ; SST:  $0.44 \pm 0.06$ ; PV:  $0.23 \pm 0.05$ ). PN vs SST p=0.94;  
 915 PN vs PV, p= 0.004; SST vs PV p= 0.04, Kruskal Wallis test with Dunn's correction **C)** Example  
 916 traces for miniature EPSCs (mEPSCs) recorded at -30mV in a PV (red) or SST (orange) INs in  
 917 control conditions and in the presence of NMDA blocker D-AP5. **D)** Average or aligned mEPSC  
 918 events recorded in both SST and PV cells under control conditions (AMPA+NMDAR), as well  
 919 as after bath application of D-AP5 (AMPA only). The subtraction between these two conditions  
 920 allowed for isolating the slow component, representing the contribution of NMDAR alone  
 921 (NMDA). Summary plot of the NMDAR- and AMPAR-mediated components of mEPSCs

922 recorded in SST- and PV-INs determined using the subtraction described on the left. The data are  
923 presented as average  $\pm$  SEM.





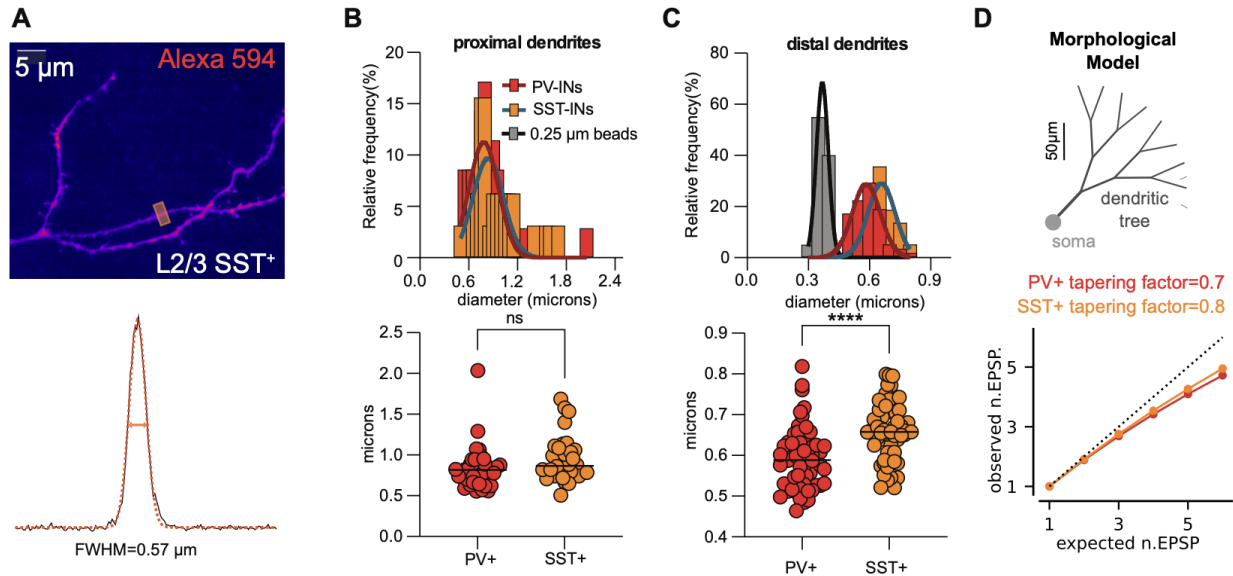
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926 **Figure S3: Experimental estimation of single quanta EPSC (qEPSC) amplitude for proximal**  
 927 **and distal dendritic locations in SST- and PV-INs.** A) Graphical representation of the  
 928 experiment used to monitor qEPSC at different dendritic locations. Quantal responses were  
 929 obtained by local perfusion of a hypertonic sucrose solution placed at proximal or distal dendritic  
 930 locations using two-photon imaging (see Methods). **B) Top:** Example traces of recorded EPSCs  
 931 during a 3 sec puff application of sucrose. **Bottom:** Magnification of recorded segment showing  
 932 the sucrose evoked events in SST and PV cells. **C)** Example traces of detected and aligned sucrose-  
 933 evoked EPSCs. Average mEPSC is shown in color. **Right:** Quantification of the amplitude of  
 934 average mEPSCs obtained for proximal (square) and distal (circle) dendritic locations in both SST  
 935 (orange) and PV cells (red) plotted in function of the rise time. The values are reported as color-  
 936 coded for single experiments with the average and SEM in black (SST proximal:  $34.59 \pm 4.40$  pA;  
 937 SSTdistal:  $20.15 \pm 2.18$  pA; PVproximal:  $31.66 \pm 1.74$  pA; PVdistal:  $17.21 \pm 0.83$  pA). **D)**  
 938 Quantification of rise time and amplitude measured after excluding fast events from distal PV  
 939 recordings using 2SD of the rise time obtained from proximal recordings (see Methods). Plots are  
 940 presented as mean  $\pm$  SEM with change in single value reported as a horizontal bar (rise time all:  
 941  $0.45 \pm 0.05$ ; selected:  $0.59 \pm 0.04$ ,  $p=0.01$  Wilcoxon test; Amplitude all:  $17.69 \pm 0.84$ ; amplitude  
 942 selected:  $17.16 \pm 0.82$ ,  $p=0.30$ , Wilcoxon test.  
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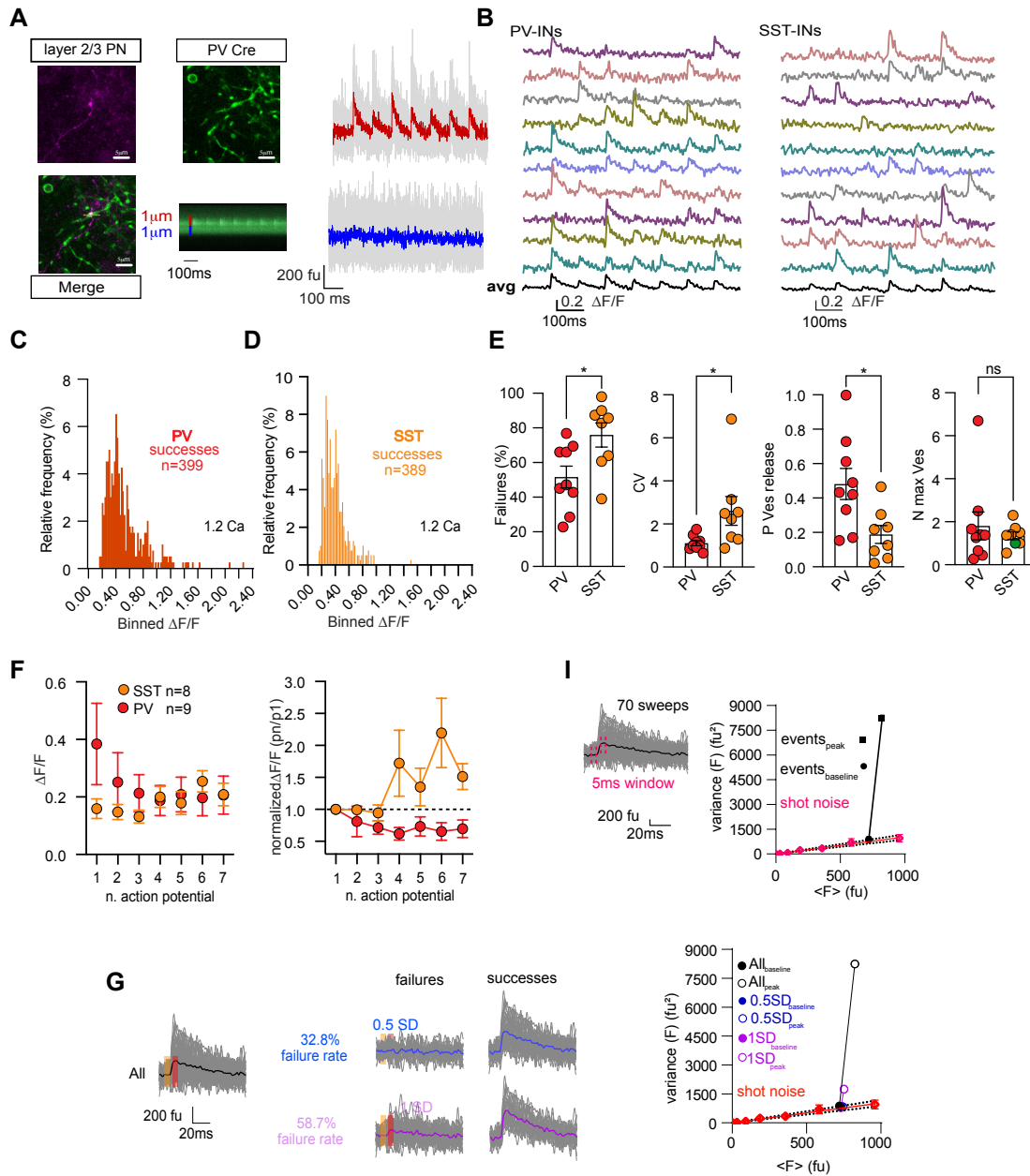
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Figure S4



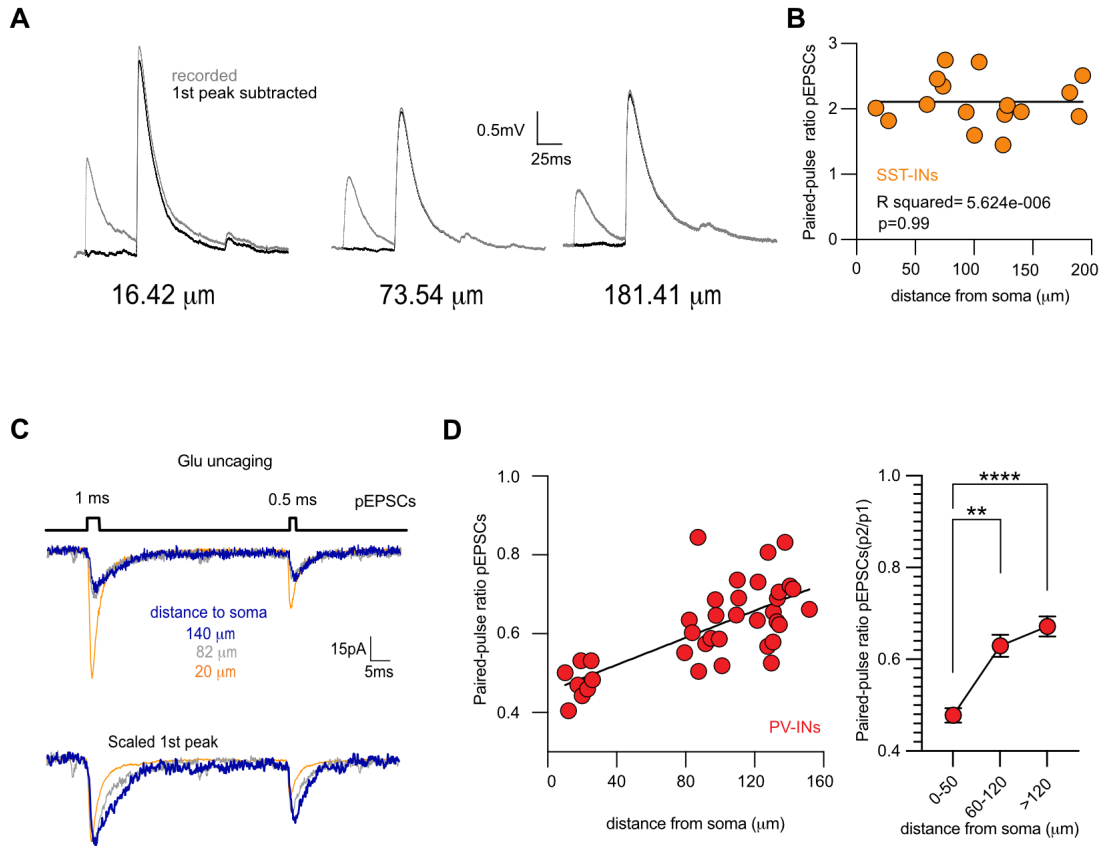
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948 **Figure S4: Dendritic diameters can not explain the qualitative difference in dendritic**  
949 **integration properties between PV- and SST-INs. A)** Example of a 2P image capturing SST-  
950 INs distal dendritic branches. The orange square defined the dendritic location used to create the  
951 intensity profile shown and quantified below (see methods). **B) Top:** Distribution of dendrite  
952 diameters for proximal (<40 $\mu\text{m}$ ) PV- (red) and SST-INs (orange) dendritic segments. *bottom:*  
953 Summary plot of average proximal dendrite diameters obtained for PV and SST-INs . The data are  
954 presented as individual values and population median (black line). PV median:0.82 mm, n=35;  
955 SST median:0.87 mm, n=32; p=0.07 Mann Whitney test. **C) Top:** Same as in B but for distal  
956 (>100 $\mu\text{m}$ ) dendritic locations. In gray the distribution for the detected diameter of 0.25  $\mu\text{m}$  beads,  
957 used to inform about lateral resolution of the 2P system. *Bottom:* Same than in **B** but for distal  
958 dendritic locations. The data are presented as single values and the median (black line). PV median:  
959 0.59 mm, n=58; SST median: 0.66 mm, n=59; p<0.0001 Mann Whitney test. **D)** Variations of  
960 dendritic diameters in the range of the experimentally-measured differences of **C** predicts minor  
961 impact on the dendritic integration mode in the simplified morphological model (see Methods).  
962 The root diameter of the morphological model is kept identical between the PV and SST model  
963 following the data of **B**. The tapering factor (the factor between the diameter of the children  
964 branches and the parent branch) was increased by 12% following the data of **C**. The average  
965 response over the 4 locations shown in Figure 1I is shown. The change in non-linearity between  
966 the PV and the SST morphologies is 2.3%.



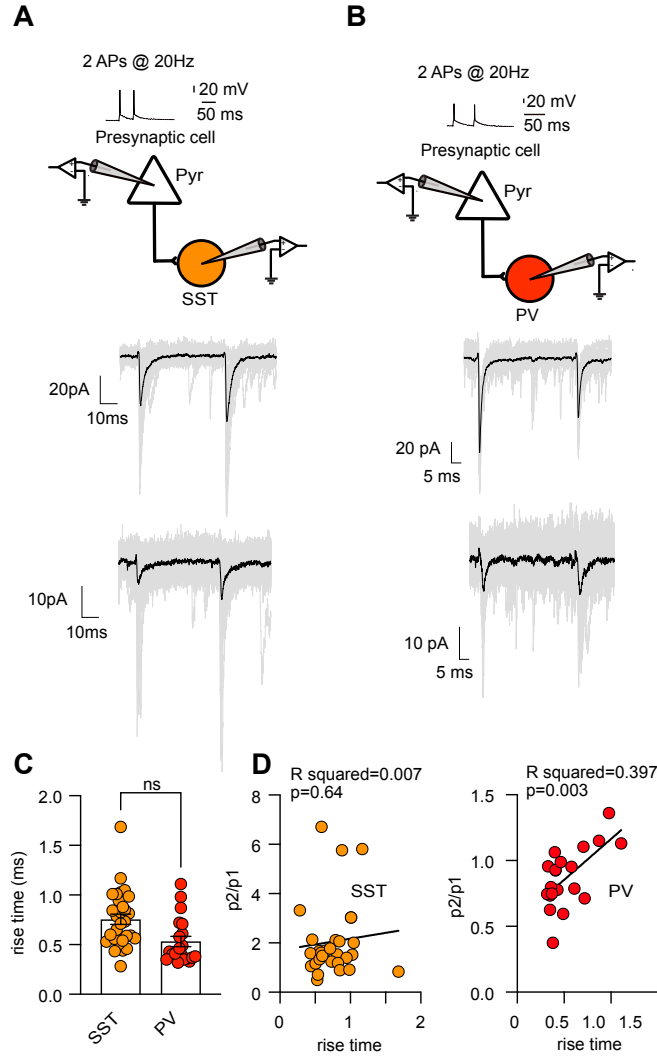
969 **Figure S5: Quantification and analysis of iGluSnFR fluorescence events.** A) Upper left: MIP  
 970 of a L2/3 PN axon patch loaded with Alexa 594. Upper middle: Positive iGluSnFR dendrites  
 971 present in the same field of view as the image on the left. iGluSnFR was selectively expressed in  
 972 PV-INs using PV-Cre transgenic mice. Bottom left: Point of contact between the Alexa 594  
 973 fluorescence (axon) and iGluSnFR signal (putative PV-dendrite) where release of glutamate was  
 974 monitored using 2P linescan. Bottom middle: Average 2P linescan (10 sweeps) obtained from point  
 975 of contact illustrated in merged image. The increase in iGluSnFR fluorescence observed upon  
 976 presynaptic firing of the individual L2/3 PYRs is restricted to a narrow (~ 1 μm) dendritic space.  
 977 Right: Time series traces are mean fluorescence of individual images over 1 μm (red and blue

978 line), and the trace is the average of all 10 traces. **B)** Single trial iGluSnFR fluorescence signal  
979 after conversion to DF/F in a PV(red) and a SST(orange) postsynaptic dendrite in the presence of  
980 1.2 mM extracellular  $\text{Ca}^{2+}$ . In black the average of 10 sweeps. **C)** Histogram of DF/F amplitude  
981 distributions of all iGluSnFR events detected in PV(orange) recorded at 1.2 mM extracellular  
982  $\text{Ca}^{2+}$ (bin value=0.03 DF/F). **D)** Same as **C** but for SST dendrites. **E)** Summary plots of failure rates  
983 and coefficient of variation obtained from iGluSnFR imaging in 1.2 mM extracellular calcium for  
984 PV- ( $n = 9$ ) and SST- ( $n = 8$ ) INs. Release probability and number of release vesicles per action  
985 potential were calculated using optical fluctuation analysis in the 1.2 mM  $\text{Ca}^{2+}$  condition assuming  
986 a binomial distribution model. The data are presented as mean $\pm$ SEM (Failures PV: 51.64 $\pm$ 6.30%;  
987 SST: 75.94 $\pm$ 6.94%,  $p=0.04$ ; CV PV: 1.11 $\pm$ 0.11; SST: 2.61 $\pm$ 0.66,  $p=0.01$ ; P Ves release PV:  
988 0.48 $\pm$ 0.09; SST: 0.19 $\pm$ 0.05,  $p=0.01$ ; N max Ves PV: 1.80 $\pm$ 0.65; SST: 1.35 $\pm$ 0.19,  $p=0.91$ . Mann-  
989 Whitney test. **F) Left:** Mean  $\pm$  SEM of the DF/F values obtained per AP. *Right:* normalized DF/F  
990 values from the experiments on the right. **I)** Example of iGluSnFR fluorescence recorded for 70  
991 APs (grey). Average trace is shown in black. The dotted line represents the time interval (5ms)  
992 and the location in the events used for measuring baseline and peak fluorescence values. On the  
993 right the graphical representation of the mean-variance plot for the baseline (black circle) and the  
994 peak fluorescence values (black). The magenta circle represents the mean-variance plot obtained  
995 by imaging the patch pipette at different laser powers to estimate variance expected from shot  
996 noise. In dotted black the 99% confidence interval for the measurement (see methods). **G) Left:**  
997 Example of iGluSnFR fluorescence signals recorded for 70 APs (grey) evoked in a single  
998 presynaptic L2/3 PYRs. Average response is shown in black. The two colored 5 ms windows  
999 represent the location for the baseline and peak fluorescence measurements. *Center:* Example of  
1000 failure rate detection obtained using two different amplitude thresholds. The success detection was  
1001 performed using a thresholding method comparing amplitude in peak window + SD of baseline  
1002 (baseline average $\pm$ 0.5 or 1 SD of the baseline; see methods). Different multiple factors of SD were  
1003 used and best value was chosen using mean-variance plots like illustrated in right. *Right:* Mean-  
1004 variance plot for baseline and peak windows on the traces illustrated in the left using baseline $\pm$  0.5  
1005 or 1 SD. The empty circles represent the peak values and the full circle the baseline. Based on the  
1006 peak variance detection the value to be used for the failure detection is baseline  $\pm$  0.5 SD. In this  
1007 case the experiment presents a failure rate of 32.8%.



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1010 **Figure S6: Integration of single and double quanta in SST+ dendrites in the current-clamp**  
 1011 **configuration and Double quanta integration in PV+ IN dendrites mimicking short-term**  
 1012 **depression** **A)** Example pEPSP traces of single and double quanta integration, separated by 50 ms  
 1013 (in gray), along an SST+ IN dendrite. In black the isolated second pEPSPs after subtraction of the  
 1014 first evoked response. **B)** Quantification of double quanta integration at different dendritic  
 1015 locations (n=16) expressed as paired-pulse ratio. In black the linear fit. **C) Top:** Double and single  
 1016 quanta integration mimicking a paired pulse depression at different dendritic locations (color  
 1017 coded). **Bottom:** Normalized traces scaled for the single quanta (2<sup>nd</sup> peak). **D) (left)** Quantification  
 1018 of double quanta integration at different dendritic locations by using paired pulse ratio (n=37). In  
 1019 black the linear fit ( $p < 0.0001$   $R^2 = 0.49$ ). **(right)** Histogram of binned PPR for values reported in B.  
 1020 Data are presented as mean  $\pm$  SEM (0-50:  $0.48 \pm 0.01$ ; 60-120:  $0.63 \pm 0.02$ ; >120:  $0.67 \pm 0.02$ . 0-50 vs  
 1021 60-120  $p = 0.002$ ; 0-50 vs >120  $p < 0.0001$ , Kruskal Wallis test with Dunn's correction.



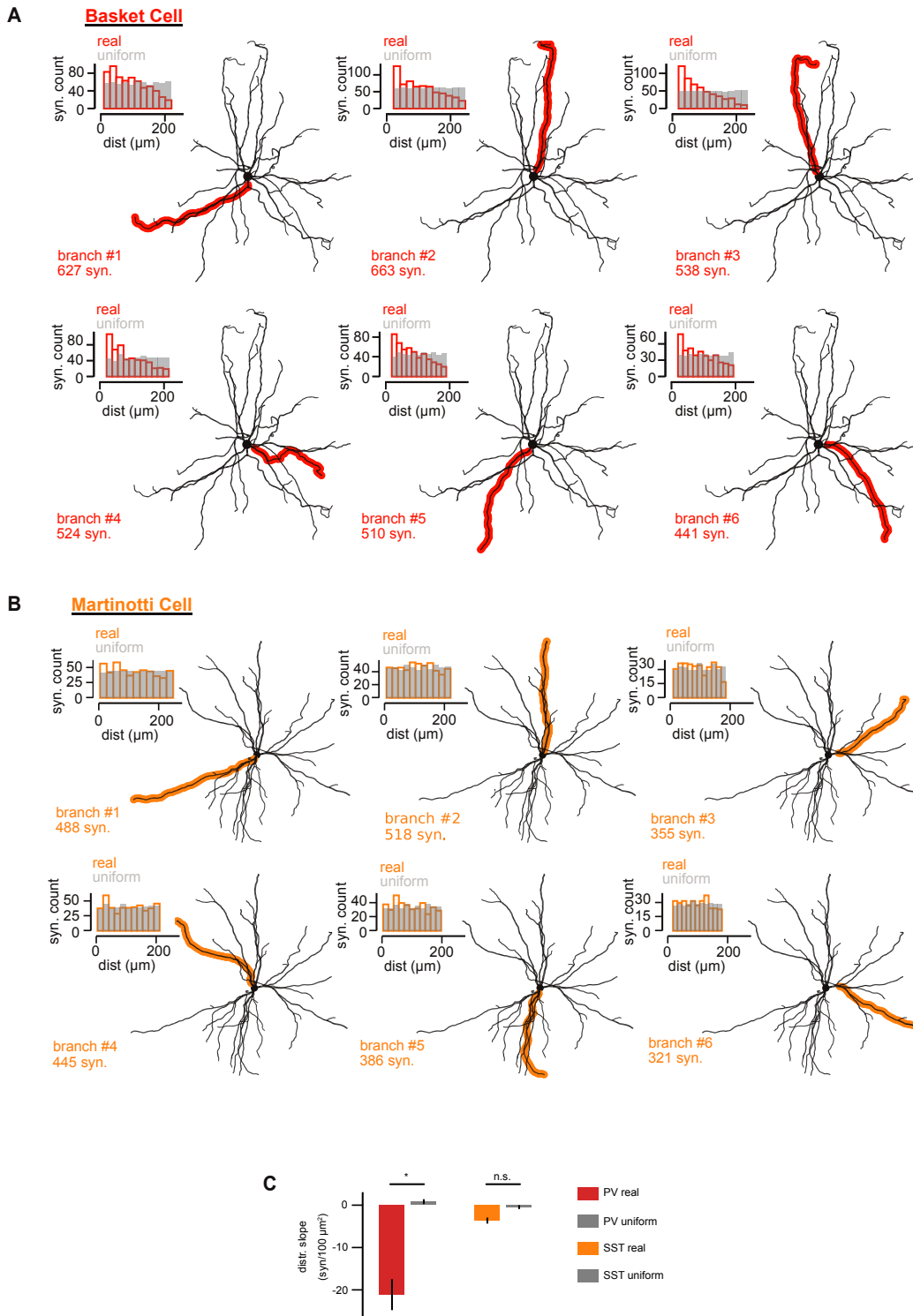
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1024 **Figure S7: Paired recordings suggest a distance-dependent gradient of short-term plasticity**  
 1025 **in PV-INs but not SST-INs. A)** (top) Illustration of dual whole-cell patch clamp recording used  
 1026 to probe unitary synaptic connections between L2/3 PNs and SST-INs. (bottom) Two example  
 1027 traces of EPSCs recorded in response to a paired-pulse stimulation in a single L2/3 PN. The two  
 1028 examples differ in rise time and amplitude. **B)** Same as in **A** but for unitary connections onto PV-  
 1029 INs. **C)** Summary data plot of EPSC rise time (first peak) recorded in SST(orange) and in PV(red)  
 1030 IN. Data are presented as mean±SEM (SST: 0.66±0.05 n=37; PV: 0.53±0.06 n=17, p=0.06 Mann  
 1031 Whitney test). **D)** Summary plot of paired pulse ratios in function of rise time for the different  
 1032 recorded synaptically connected cell pairs. The black lines represent the linear fit (SST p=0.64,  
 1033 R<sup>2</sup>=0.007; PV p=0.03, R<sup>2</sup>=0.39).

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Figure S8



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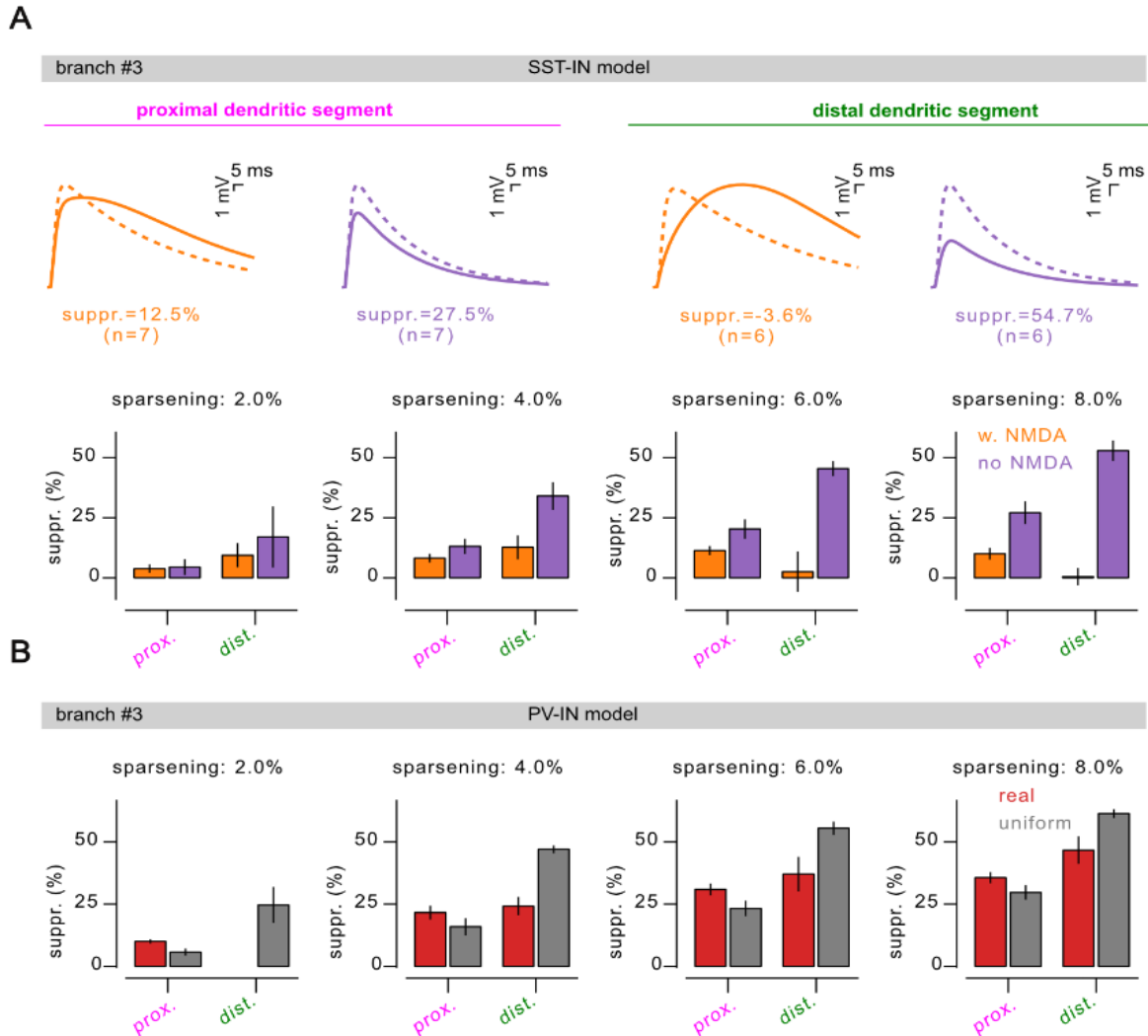
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**Figure S8: Dendritic branches used for numerical simulations in the PV-IN and SST-IN models. A) Morphology of a V1 basket cell reconstructed from serial electron microscopy imaging**

1039 highlighting the six distinct dendritic branches used for numerical simulations of the PV-IN model.  
1040 Histogram (top inset) of the real synapse distribution (colored) and its uniform-density surrogate  
1041 (gray, see Methods) for the different individual dendritic branches. **B)** Same as **A** but for the  
1042 Martinotti Cell used in the SST-IN model. **C)** Distributions slopes (of the synaptic count vs  
1043 distance to soma) in the real synaptic distributions and uniform surrogates. Basket cell, real: -  
1044  $21.1 \pm 3.7$  syn./100 $\mu\text{m}$ , uniform:  $0.8 \pm 0.6$  syn./100 $\mu\text{m}$ , n=6 branches, p=3e-2, Wilcoxon test.  
1045 Martinotti cell, real:  $-3.6 \pm 0.7$  syn./100 $\mu\text{m}$ , uniform:  $-0.5 \pm 0.5$  syn./100 $\mu\text{m}$ , n=6 branches, p=6e-2,  
1046 Wilcoxon test.  
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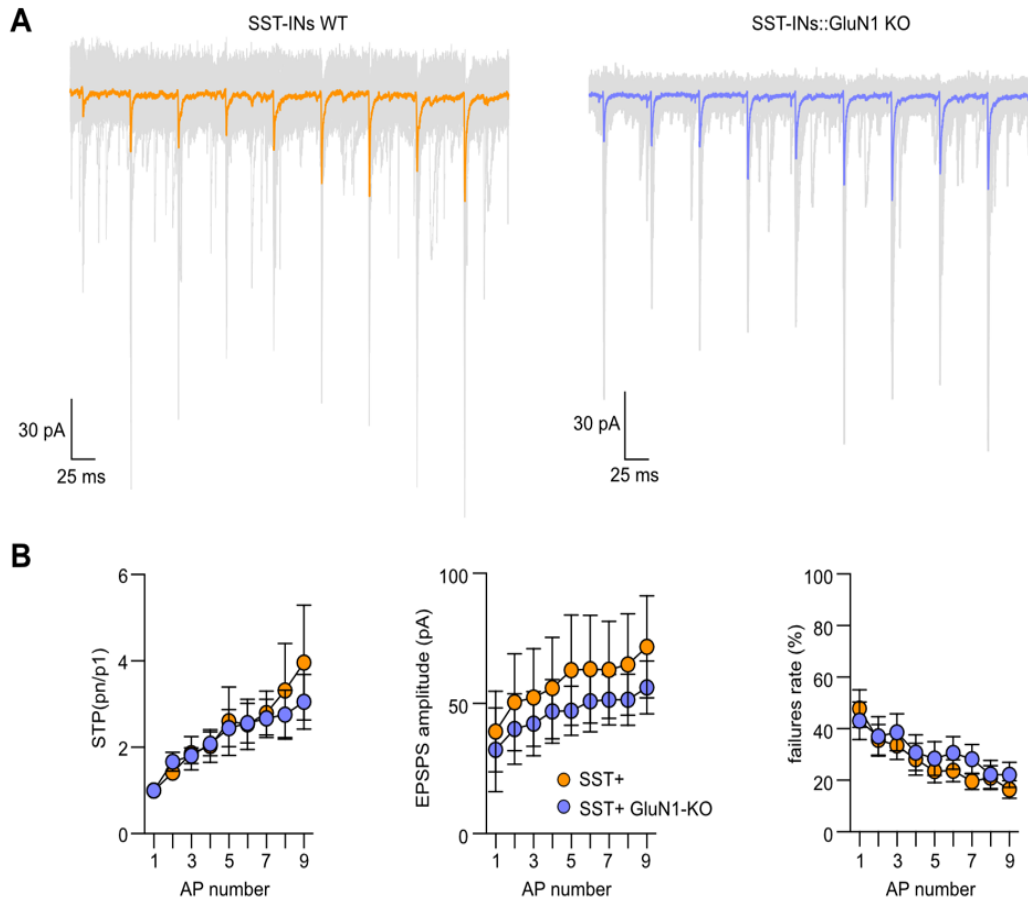


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**Figure S9: Distal and proximal integration upon increasing number of co-active inputs in dendritic segments of the morphologically-detailed interneuron models.** Related to Fig. 4. We vary the sparsening variable that sets synaptic recruitment from 2% (left) to 8% (right) in the PV-model (A) and SST-model (B) with their respective control (see main text, “uniform” distribution for the PV-model and “no-NMDA” setting for the SST-model. Note the very high level of suppression (>50%) for sparsening above 4% in the PV-model. Note also the appearance of supra-linear integration (suppression <0%) in the distal segment of the SST-model in presence of NMDAR at a sparsening of 8%. C) Example  $V_m$  response in a branch in the SST-model at a sparsening of 8%. Integration from the distal segment stimulation exhibits supra-linear integration (suppression <0%).

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Figure S10



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1064 **Figure S10: Synaptic transmission between L2/3 PN and L2/3 SST-INs is not altered in**  
1065 **SST::GluN1 KO mice. A)** Representative traces of uEPSCs recorded in a postsynaptic L2/3 SST-  
1066 IN held at -70 mV in WT (left) and in SST-Cre::GluN1 flox mice after a train of action potentials  
1067 (9 @ 40 Hz) induction in a presynaptic L2/3 pyramidal neuron (PN). Single repetitions (30 sweeps)  
1068 are in faint gray, average is in full color. **(B)** Summary plot of short-term plasticity, uEPSCs  
1069 amplitude and failures rate (first EPSC of the train of stimulation) for the unitary connection  
1070 between L2/3 PN and L2/3 SST-INs in WT (n = 14) and in SST-Cre::GluN1 flox mice (n = 9).

1071 **Supplementary Tables**

1072

<b>Supp. Table 1. Passive properties of interneuron models.</b>				
<b>Property</b>	<b>Soma</b>	<b>Proximal dendrite</b>	<b>Distal dendrite</b>	<b>Constraint/Reference</b>
<i>PV model</i>				
Leak conductance ( $g_{pas}$ )	$4.37 \cdot 10^{-4}$ S.cm <sup>-2</sup>	$4.37 \cdot 10^{-4}$ S.cm <sup>-2</sup>	$4.46 \cdot 10^{-5}$ S.cm <sup>-2</sup>	Adjusted to get a somatic input resistance of 100M $\Omega$ while keeping the prox./dist ratio of (11).
Resting Membrane Potential ( $e_{pas}$ )	-70 mV	-70 mV	-70 mV	(56, 57)
Membrane capacitance ( $cm$ )	1.2 $\mu$ F.cm <sup>-2</sup>	1.2 $\mu$ F.cm <sup>-2</sup>	1.2 $\mu$ F.cm <sup>-2</sup>	(58)
Axial Resistance ( $Ra$ )	172 $\Omega$ .cm	142 $\Omega$ .cm	142 $\Omega$ .cm	(58)
<i>SST model</i>				
Leak conductance ( $g_{pas}$ )	$3.5 \cdot 10^{-5}$ S.cm <sup>-2</sup>	$3.5 \cdot 10^{-5}$ S.cm <sup>-2</sup>	$3.5 \cdot 10^{-5}$ S.cm <sup>-2</sup>	Adjusted to get a somatic input resistance in 300 +/- 40 M $\Omega$ (56), here 285M $\Omega$
Resting Membrane Potential ( $e_{pas}$ )	-60 mV	-60 mV	-60 mV	(60, 61)
Membrane capacitance ( $cm$ )	1.2 $\mu$ F.cm <sup>-2</sup>	1.2 $\mu$ F.cm <sup>-2</sup>	1.2 $\mu$ F.cm <sup>-2</sup>	(58)
Axial Resistance ( $Ra$ )	172 $\Omega$ .cm	142 $\Omega$ .cm	142 $\Omega$ .cm	(58)

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<b>Supp. Table 2. Active current densities in the interneuron models.</b>				
<b>Current</b>	<b>Soma</b>	<b>Proximal dendrite</b>	<b>Distal dendrite</b>	<b>Constraint/Reference</b>
<i>PV model</i>				
Fast Sodium current	$3.4 \cdot 10^{-1}$ S.cm <sup>-2</sup>	0 S.cm <sup>-2</sup>	0 S.cm <sup>-2</sup>	Adjusted to match rheobase in 216 +/- 40 pA (59), here 229.3 pA
Delayed rectifier Potassium current	$9.60 \cdot 10^{-2}$ S.cm <sup>-2</sup>	0 S/cm <sup>-2</sup>	0 S.cm <sup>-2</sup>	Keeping ratio with sodium current of (11).
A-type Potassium current (proximal)	0 S.cm <sup>-2</sup>	$1.0 \cdot 10^{-3}$ S.cm <sup>-2</sup>	0 S.cm <sup>-2</sup>	(11).
A-type Potassium current (distal)	0 S.cm <sup>-2</sup>	$9.0 \cdot 10^{-4}$ S.cm <sup>-2</sup>	$2.2 \cdot 10^{-3}$ S.cm <sup>-2</sup>	(11).
<i>SST model</i>				

Fast Sodium current	$7.0 \cdot 10^{-1}$ S.cm <sup>-2</sup>	0 S/cm2	0 S/cm2	Adjusted to match rheobase 76.9 +/- 35 pA (60), here 93.1pA.
Delayed rectifier Potassium current	$4.0 \cdot 10^{-1}$ S.cm <sup>-2</sup>	0 S/cm2	0 S/cm2	Keeping ratio with sodium current of (11).
M-type Potassium current	$2.0 \cdot 10^{-3}$ S.cm <sup>-2</sup>	0 S/cm2	0 S/cm2	

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<b>Supp. Table 3. Synaptic properties of the interneuron models.</b>			
<b>Receptor</b>	<b>Quantal</b>	<b>Decay</b>	<b>Constraint/Reference</b>
<b><i>PV model</i></b>			
AMPA	0.8 nS	2 ms	(61)
GABA	2 nS	5.5 ms	(61)
<b><i>SST model</i></b>			
AMPA	0.8 nS	2 ms	(61)
NMDA	1.2 nS	80 ms	(61) Constrained from NMDA/AMPA ratio=1.5
GABA	2 nS	5.5 ms	(61)

1076

1077 **Methods**

1078 **Mice**

1079 Animals (C57BL/6 background) were housed in the Paris Brain Institute animal facility accredited  
1080 by the French Ministry of Agriculture for performing experiments on live rodents under normal  
1081 light/dark cycles. Work on animals was performed in compliance with French and European  
1082 regulations on care and protection of laboratory animals (EC Directive 2010/63, French Law  
1083 2013–118, February 6th, 2013). All experiments were approved by local the Ethics Committee  
1084 #005 and by French Ministry of Research and Innovation. Experimental data was obtained from  
1085 adult (P25-P70) mice. Both male and female mice were used with the following genotypes: SST-  
1086 IRES-Cre (SSTtm2.1(cre)Zjh/J; JAX 013044) X Ai9 (Gt(ROSA)26Sortm9(CAG-tdTomato); JAX  
1087 007909); PV-Cre ( Pvalbtm1(cre)Arbr/J; JAX 008069) X Ai9 (Gt(ROSA)26Sortm9(CAG-  
1088 tdTomato); SST-IRES-Cre (SSTtm2.1(cre)Zjh/J; JAX 013044) X PSD-95-ENABLED (B6;129-  
1089 Dlg4tm1.1Hnz/J; JAX 026092); PV-Cre ( Pvalbtm1(cre)Arbr/J; JAX 008069) X PSD-95-  
1090 ENABLED (B6;129-Dlg4tm1.1Hnz/J; JAX 026092). Animals were maintained on a 12-h  
1091 light/dark cycles with food and water provided ad libitum.

1092

1093 **Slice preparation**

1094 Acute parasagittal slices (320  $\mu$ m) were prepared from adult mice, aged between postnatal days  
1095 25 and 70. Mice were deeply anesthetized with a mix of ketamine/xylazine (mix of i.p. ketamine  
1096 100 mg/kg and xylazine 13 mg/kg) and perfused transcardially with ice-cold cutting solution  
1097 containing the following (in mM): 220 sucrose, 11 glucose, 2.5 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>,  
1098 7 MgSO<sub>4</sub>, 0.5 CaCl<sub>2</sub>. After the perfusion the brain was quickly removed and slices prepared using  
1099 a vibratome (Leica VT1200S). Slices containing S1 barrel field were transferred to ACSF solution  
1100 at 34°C containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>,  
1101 15 Glucose for 15–20 min. After the period of recovery, slices were kept at room temperature for  
1102 a period of 5-6 h.

1103

1104 **Electrophysiology**

1105 Whole-cell patch-clamp recordings were performed close to physiological temperature (32–34°C)  
1106 using a Multiclamp 700B amplifier (Molecular Devices) and fire-polished thick-walled glass patch  
1107 electrodes (1.85 mm OD, 0.84 mm ID, World Precision Instruments); 3.5–5 MOhm tip resistance.  
1108 For voltage-clamp recordings, cells were whole-cell patched using following intracellular solution  
1109 (in mM): 90 Cs-MeSO<sub>3</sub>, 10 EGTA, 40 HEPES, 4 MgCl<sub>2</sub>, 5 QX-314, 2.5 CaCl<sub>2</sub>, 10  
1110 Na<sub>2</sub>Phosphocreatine, 0.3 MgGTP, 4 Na<sub>2</sub>ATP (300 mOsm pH adjusted to 7.3 using CsOH).  
1111 Extracellular synaptic stimulation was achieved by applying voltage pulses (20  $\mu$ s, 5–50 V;  
1112 Digitimer Ltd, UK) via a second patch pipette filled with ACSF and placed 20–40  $\mu$ m from soma.  
1113 For current clamp glutamate uncaging experiments patch pipettes were filled with the following  
1114 intracellular solution (in mM): 135 K-gluconate, 5 KCl, 10 HEPES, 0.01 EGTA, 10  
1115 Na<sub>2</sub>phosphocreatine, 4 MgATP, 0.3 NaGTP (295 mOsm, pH adjusted to 7.3 using KOH). The  
1116 membrane potential (V<sub>m</sub>) was held at -60 mV, if necessary, using small current injection (typically  
1117 in a range between -50 pA and 200 pA). Recordings were not corrected for liquid junction  
1118 potential. Series resistance was compensated online by balancing the bridge and compensating  
1119 pipette capacitance. For glutamate uncaging experiments Alexa 594 (20  $\mu$ M) was added to the  
1120 intracellular solution daily. In all experiments data were discarded if series resistance, measured  
1121 with a -10 mV pulse in voltage clamp configuration, was >20 M $\Omega$  or changed by more than 20%

1122 across the course of an experiment. For current clamp experiment cells were excluded if input  
1123 resistance varied by more than 25% of the initial value. AMPA-EPSCs were recorded at -70 mV  
1124 in the presence of the GABAA blocker picrotoxin (100  $\mu$ M, Abcam). To record NMDA-EPSCs,  
1125 NBQX (10  $\mu$ M, Tocris) was added to the ASCF and the resting membrane potential changed to  
1126 +40 mV. All recordings were low-pass filtered at 10 kHz and digitized at 100 kHz using an analog-  
1127 to-digital converter (model NI USB 6259, National Instruments, Austin, TX, USA) and acquired  
1128 with Nclamp software (62) running in Igor PRO (Wavemetrics, Lake Oswego, OR, USA). For all  
1129 the experiments L2/3-PV+-INs and L2/3-SST+-INs were identified using PV-Cre and SST-cre  
1130 mice crossed with the reporter mouse line (Ai9, (ROSA)26Sortm9 (CAG-tdTomato)Hze/Jt; JAX  
1131 007909). The intracellular solution used for the experiments in which a presynaptic cell was  
1132 depolarized by a train of action potentials to induce neurotransmitter release on a post-synaptic  
1133 cell/element was (in mM): 130 K-MeSO<sub>3</sub>, 4 MgCl<sub>2</sub>, 10 HEPES, 0.01 EGTA, 4 Na<sub>2</sub>ATP, 0.3  
1134 NaGTP (300 mOsm, pH 7.3 adjusted with NaOH). Paired recordings connections were probed  
1135 using a train of 5/9 action potentials at frequency of 20 Hz. APs were initiated by brief current  
1136 injection ranging from 1000–2000 pA and 1–5 ms duration. After a clear identification of synaptic  
1137 connection (mean of 30 sweeps with clear AMPA current), post-synaptic cell membrane potential  
1138 was brought from the initial -70 mV membrane potential to +40 mV to record the NMDAR-EPSCs  
1139 in the presence of blockers as described before. To visualize single boutons in the experiments  
1140 targeting SFiGluSnFR.A184V positive dendritic elements, we added 40  $\mu$ M Alexa 594 in the  
1141 intracellular solution.  
1142

#### 1143 **Transmitted light and fluorescence imaging**

1144 Neurons were visualized using infrared Dodt contrast (Luigs and Neumann, Ratingen, Germany)  
1145 and a frame transfer CCD camera (Infinity-Lumenera). These components were mounted on an  
1146 Ultima two-photon laser scanning head (Bruker, USA) based on an Olympus BX61W1  
1147 microscope, equipped with a water-immersion objective (60x, 1.1 numerical aperture, Olympus  
1148 Optical, Japan). The somas of somatostatin (SST) and parvalbumin (PV) interneurons were  
1149 identified using a td-Tomato reporter mouse line (see animals) combined with fluorescence  
1150 imaging. Two-photon excitation was performed with a pulsed Ti:Sapphire laser (DeepSee,  
1151 Spectra-Physics, France) tuned to 810 or 840 nm for imaging neuronal morphology. Individual  
1152 neurons were patch-loaded with Alexa 594 (20  $\mu$ M). In some instances, we used a transmitted light  
1153 PMT mounted after the Dodt tube to acquire a laser-illuminated contrast image simultaneously  
1154 with the 2PLSM image. This dual imaging mode was used to position stimulation electrodes of  
1155 sucrose puffing pipettes close to a spatially isolated dendrite, identified from maximal intensity  
1156 projections of 2PLSM images. In addition to the light collected through the objective, the  
1157 transmitted infrared light was collected through a 1.4 NA oil-immersion condenser (Olympus),  
1158 and reflected on a set of substage photomultiplier tubes (PMTs). SFiGluSnFR.A184V fluorescence  
1159 were filtered using hq525/70 nm bandpass filters (Chroma) and detected with gallium arsenide  
1160 phosphate-based PMTs (H10770PA-40, Hamamatsu Photonics), and Alexa Fluor 594  
1161 fluorescence were filtered using hq607/45 bandpass filters (Chroma) and detected using gallium  
1162 arsenide phosphate-based PMTs (H10770PA-40, Hamamatsu Photonics). 200 nm yellow-green  
1163 fluorescent beads (Life Technologies) were used to estimate the point spread function (PSF) of the  
1164 microscope system. The measured PSF at 810 nm had lateral dimensions of  $368 \pm 5.19$  nm (full  
1165 width at half maximum,  $n = 20$ ).  
1166

1167 **Stereotaxic viral injections**

1168 Adult mice were bilaterally injected in the primary somatosensory cortex barrel field (S1-BF) by  
1169 or unilaterally in the primary visual cortex (V1) using a 10  $\mu$ l Hamilton syringe (1701RN; Phymep,  
1170 France) with a borosilicate glass capillary (O.D. 1.0mm; I.D.0.50mm; 10 cm length item #: BF100-  
1171 50-10 WPI) pulled and beveled mounted on automatic injector used to infuse between 100 and  
1172 450 nl of virus at a rate of 60 nl/min using the following coordinates relative to Bregma: S1  
1173 anterior-posterior [AP], -0.5mm; medial lateral [ML],  $\pm$  3.2 mm and dorso ventral [DL] -0.2 mm  
1174 relative to brain surface; V1(anterior-posterior [AP], -4.1mm; medial lateral [ML],  $\pm$  2.8 mm and  
1175 the dorso ventral [DL], -0.2 mm relative to brain surface. Following the injection, the beveled glass  
1176 capillar was left in place for 4 minutes to allow full diffusion of the viruses. *Ex vivo*  
1177 electrophysiology experiments were performed at least 2/3 weeks after the surgery. For monitoring  
1178 glutamate release at individual glutamatergic synapses, PV- or SST-cre mice were stereotaxically  
1179 injected with AAV9.CAG-FLEX.SFiGluSnFR.A184V (Addgene plasmid #106205; (39)). For in  
1180 vivo two photon calcium imaging mice were injected with AAV9 Syn-Flex-GCaMP6s-WPRE-  
1181 SV40 (2.5x10<sup>13</sup> AddGene-100845). To trace dendritic elements from SST or PV positive neurons  
1182 in the STED experiment we inject AAV1.Flex.tdTomato (Addgene-28360) in S1-BF.

1183

1184 **Estimation quantal miniature EPSCs**

1185 The morphology of L2/3 SST- and PV-INs was visualized using fluorescence imaging of patch-  
1186 loaded Alexa 594 (20  $\mu$ M). The extracellular solution was modified to contain in mM: 0 CaCl<sub>2</sub>  
1187 and 3 MgCl<sub>2</sub> in the presence of TTX 0.5  $\mu$ M (63). Neurons were patch loaded with Alexa 594 for  
1188 at least 20 min to visualize cell morphology using two-photon microscopy with a laser tuned to  
1189 820 nm and modulated using a Pockels cell (Conoptics, Danbury, CT). This strategy allowed us  
1190 to identify the proximal and distal dendrites of the patched neuron. A puff pipette was then placed  
1191 at 20-30  $\mu$ m from the selected dendrite where an extracellular hypertonic sucrose solution (350  
1192 mOsm) was puffed for 2 seconds at 0.40 bar positive pressure. Single evoked miniature events  
1193 were then isolated from the 2 second sucrose puffing period recorded in 20 sweeps. On average  
1194 120 events were used for analysis. For single experiments rise time estimation was performed by  
1195 fitting single events with a triexponential function. Fitted events have been subsequently aligned  
1196 for their respective 10% rise time which finally resulted in the reported amplitude and kinetics  
1197 values (fig S3). To reduce possible contamination of distally recorded events by spontaneous  
1198 proximal events in PV-INs due to their elevated basal rate of mEPSCs we used an exclusion criteria  
1199 based on rise times. During estimation of sucrose evoked mEPSCs in distal dendritic regions in  
1200 PV-INs we excluded events whose rise time was within mean+2SD the rise time of the sucrose  
1201 mEPSCs obtained on the same cell. This criteria allowed us to remove spontaneous proximal  
1202 events that occurred during the sucrose puffing period. The distance used for proximal and distal  
1203 evoked events was respectively  $13.55 \pm 3.46$  and  $113.6 \pm 4.89$  nm for PV-INs and  $22.51 \pm 2.60$   
1204 and  $124.2 \pm 9.61$  nm for SST-INs.

1205

1206 **Glutamate uncaging**

1207 L2/3 PN and L2/3 INs morphology was visualized using fluorescence imaging of patch-loaded  
1208 Alexa 594 (20  $\mu$ M). The output of two pulsed Ti:Sapphire (DeepSee, Spectra Physics) lasers were  
1209 independently modulated to combine uncaging of MNI-glutamate and Alexa 594 imaging. The  
1210 imaging laser beam was tuned to 820 nm and modulated using a Pockels cell (Conoptics, Danbury,  
1211 CT). For uncaging, the intensity and duration (0.5s or 1s for single and double quanta experiment)  
1212 of illumination of the second Ti:Sapphire laser tuned to 720 nm was modulated using an acousto-

1213 optic modulator (AA Opto-Electronic, France). MNI-caged-L-glutamate (10 mM) was dissolved  
1214 in a solution containing (in mM): NaCl 125, glucose 25, KCl 2.5, HEPES 10, CaCl<sub>2</sub> 2 and MgCl<sub>2</sub>  
1215 1 (pH 7.3) and constantly puffed using a glass pipette (res 1.5–2 MΩ) placed in the proximity  
1216 of a selected dendrite. Multiple uncaging locations (6–12) were placed adjacent to visually  
1217 identified spines for PNs or in proximity to the shafts for INs (1 μm) and not closer to each other  
1218 than 2 μm to reduce possible effect of glutamate spillover between locations. For the uncaging  
1219 experiments in INs the uncaging spots were placed along the dendritic shaft at the level of synaptic  
1220 “hot-spots” identified by single events rise time as described in (13, 64). Laser intensity was  
1221 adjusted to obtain individual pEPSP of a variable amplitude. The arithmetic sum (always corrected  
1222 for not perfect simultaneous activation) was used to compare the obtained values (expected) versus  
1223 the recorded EPSP elicited by an increasing number of inputs recruited by glutamate uncaging. An  
1224 input-output curve was obtained by plotting the amplitude of the expected EPSP versus the  
1225 amplitude of recorded EPSP. Non-linearity was calculated as previously described (65). Different  
1226 inter event time intervals were tested during the quasi simultaneous activation of inputs: 0.12 and  
1227 1, 5 or 8 ms. In a subset of experiments laser power used to evoke glutamate uncaging was adjusted  
1228 in order to obtain pEPSCs with similar amplitudes than the mean amplitude of the quantal  
1229 miniature EPSCs recorded during sucrose puff at proximal and distal locations. This initial  
1230 calibration was performed in the voltage clamp configuration. Amplitude of quantal adjusted  
1231 pEPSPs (qEPSPs) was also obtained in a separate group of experiments by shifting the recording  
1232 conditions to the current clamp configuration while maintaining uncaging location and laser  
1233 intensity. This allowed us to obtain the following values: qEPSCs PV-INs proximal=  $32.90 \pm 1.27$   
1234 pA, qEPSPs PV-INs proximal  $0.73 \pm 0.06$  mV; qEPSCs PV-INs distal=  $16.40 \pm 1.39$  pA, qEPSPs  
1235 PV-INs distal  $0.47 \pm 0.06$  mV; qEPSCs SST-INs distal=  $17.06 \pm 0.17$  pA, qEPSPs SST-INs distal  
1236  $0.83 \pm 0.17$  mV. The drugs used for uncaging experiments were placed in the puff pipette and in  
1237 the bath. In glutamate uncaging experiments involving specific receptors antagonists and/or  
1238 channel blockers, the drugs were present in both the puff pipette solution and the bath solution.  
1239 The concentration of NMDA blockers, D-AP5 and MK-801, used in the puff pipette solution was  
1240 500 μM and 50 μM respectively following the conditions described in (66). In the bath solution  
1241 concentration of D-AP5 and MK-801 used was 50 μM and 20 μM respectively. In a subset of  
1242 experiments we also blocked voltage gated calcium and sodium channels in addition to NMDARs  
1243 (described as “blockers cocktail”). For that TTX 1μM, NiCl 100μM, Nifedipine 30μM were added  
1244 to the puff and bath solution.

1245

### 1246 **iGluSnFR Imaging**

1247 Acute brain slices from animals previously injected with AAV9.CAG-FLEX.SFiGluSnFR.A184V  
1248 were performed as described above. L2/3 PNs were subsequently patch loaded with Alexa 594 (40  
1249 μM) and the axon visualized using two-photon imaging. Axonal boutons were easily identified  
1250 after 30-40 min of intracellular loading in whole cell patch-clamp configuration. Using  
1251 simultaneous two-photon imaging of Alexa 594 and iGluSnFR fluorescence signals, putative  
1252 points of contact were first identified at 820nm. The genetically encoded glutamate sensor,  
1253 iGluSnFR-A184V was selectively expressed in SST- or PV-INs. Presence of positive synaptic  
1254 connections was probed by placing a linescan (~7 μm at 0.76 ms per line) over the identified  
1255 potential synaptic connection and variation of iGluSnFR fluorescence signals (imaged at 920 nm)  
1256 monitored in response to evoked action potentials via current injection in the presynaptic L2/3 PN.  
1257 Trains of 7 action potentials (@10 Hz) repeated at a frequency 0.33Hz were used to imaged  
1258 glutamate release into PV- and SST-INs. Variations in iGluSnFR fluorescence were expressed as



1259  $\Delta F/F$  time series traces constructed from linescan images. The fluorescence as a function of time  
 1260 was averaged over visually identified pixels corresponding to width of the presynaptic bouton  
 1261 (identified using Alexa 594 fluorescence and overlapping with the iGluSnFR expressing dendrite)  
 1262 and then averaged over individual trials, resulting in a single fluorescence trace as a function of  
 1263 time ( $F_{\text{dendrite}}(t)$ ). The background fluorescence ( $F_{\text{back}}(t)$ ) was estimated similarly (identical spatial  
 1264 line length), but from a location not on a labeled structure. This  $F_{\text{back}}(t)$  trace was then fit with a  
 1265 single exponential function and then subtracted from  $F_{\text{dendrite}}(t)$  in order to correct for bleaching.  
 1266 The background corrected traces ( $F(t)$ ) was then converted to the final  $\Delta F/F(t)$  according to  
 1267 Equation 1:  
 1268

$$\frac{\Delta F}{F}(t) = \frac{F(t) - F_{\text{rest}}}{F_{\text{rest}}} \quad (1)$$

1269  
 1270 where  $F_{\text{rest}}$  is the time averaged (100 ms window) fluorescence before stimulation and  $F(t)$  is the  
 1271 time-dependent fluorescence transient  
 1272  
 1273

### 1274 **Optical fluctuation analysis**

1275 The estimation of number release sites ( $N_{\text{maxVes}}$ ) and probability of release ( $P_{\text{Ves}}$ ) from single  
 1276 boutons using iGluSnFR fluorescence signals was performed using fluctuation analysis inspired  
 1277 in the method previously described for estimating number and open probability of voltage gated  
 1278 calcium channels (VGCCs; (36, 37)). We considered simplistically that if neurotransmitter release  
 1279 at single point of contact is governed by binomial statistics, then the coefficient of variation of the  
 1280 dark/shot noise subtracted iGluSnFR fluorescence variance is given by:  
 1281

$$CV_{F, \Delta iGluSnFR} = \frac{\sqrt{\sigma_F^2 - \sigma_d^2 - \sigma_{sn}^2}}{\langle \Delta F \rangle} \quad (2)$$

1282  
 1283  
 1284 where  $\sigma_F^2$  is the total signal variance,  $\sigma_d^2$  is the dark noise and  $\sigma_{sn}^2$  represents the shot noise  
 1285 contributions, where the shot noise variance was calculated by imaging a pipette filled with Alexa  
 1286 488 with varying laser intensities. To ensure that variance was related to fluctuations in  
 1287 neurotransmitter release and to minimize other sources of variance, including optical drift we  
 1288 corrected the variance with a trial to trial correction for drift as reported in (67) by using the  
 1289 equation:  
 1290  
 1291

$$\sigma_F^2 = \frac{1}{2(n-1)} \sum_{i=1}^{n-1} (\Delta F_{i+1} - \Delta F_i)^2 \quad (3)$$

1292  
 1293 where  $n$  represents the number of trials. This normalization minimizes the variance increase given  
 1294 by a run up or run down of fluorescence during the recording. Recordings in which the baseline  
 1295 variance (calculated from a 100 ms window before AP induction) was larger than the 99%  
 1296 confidence interval of the shot noise expected variance were not included in the analysis. Failure  
 1297 rate estimation was defined using a thresholding approach on the  $F(t)$  trace. We systematically  
 1298 tested multiple different thresholds. For that we compared the mean  $F_{iGluSnFR}$  value (calculated  
 1300 from a 10 ms window from the  $F(t)$  trace just after presynaptic evoked action potentials) to the  
 1301 mean + 0.5, 1 or 2 times the SD of the baseline (prior action potential). Release successes were  
 1302 defined if  $F$  value after AP was larger than threshold. In order to optimally define the best threshold

1303 for each individual experiment we computed variance from the failure trials defined for the  
 1304 different thresholds and compared to predicted shot noise variance. All successes were further  
 1305 analyzed as  $\Delta F/F$ . To estimate the amplitude of release successes, we fit averaged  $\Delta F/F$  traces with  
 1306 the following equation (68), using a least-square algorithm implemented in IgorPro  
 1307 (Wavemetrics):

$$1308 \quad \frac{\Delta F}{F}(t) = A_1 \left( 1 - \exp\left(\frac{1-t_0}{\tau_{rise}}\right) \right)^n \left( A_2 \exp\left(\frac{t-t_0}{\tau_{decay1}}\right) \right) \left( A_3 \exp\left(\frac{t-t_0}{\tau_{decay2}}\right) \right) \quad (4)$$

1309  
 1310 Assuming that neurotransmitter release at single point of contact is governed by binomial  
 1311 statistics the probability that an action potential fails to release any synaptic vesicle is given by the  
 1312 equation:

$$1313 \quad P(0) = (1 - p)^N \quad (5)$$

1314  
 1315 where p is the release probability of individual vesicles and N is the number of release sites.  
 1316 Equation 5 together with  $CV_{F,iGluSnFR}$  can be used to solve for N and p.

$$1317 \quad P(0) = \left( \frac{CV_{F,iGluSnFR}^2}{\frac{1}{n} + CV_{F,iGluSnFR}^2} \right)^N \quad \text{and} \quad p = \frac{1}{1 + N CV_{F,iGluSnFR}^2} \quad (6, 7)$$

1318

### 1319 **PSD95 labelling in cortical interneurons.**

1320 We examined the distribution of excitatory synaptic inputs along the somatodendritic compartment  
 1321 in SST- and PV-INs using transgenic mouse lines that conditionally express Venus-tagged PSD95  
 1322 under the control of the SST- or PV- promoter (PSD95-Enabled (42)  $\times$  SST Cre or PV-Cre  
 1323 respectively. Stereotaxic viral injections of AAV1.Flex.tdTomato was used to visualize  
 1324 interneuron morphology in such transgenic mouse lines. Two/three weeks after stereotaxic viral  
 1325 infections, SST-PSD-95-CreNABLED and PV-PSD-95-CreNABLED adult mice were deeply  
 1326 anesthetized with a mix of ketamine/xylazine (mix of i.p. ketamine 100 mg/kg and xylazine 13  
 1327 mg/kg) and perfused transcardially with ice-cold PBS 1X (ET330-A) followed by ice-cold PFA  
 1328 4% freshly prepared. At least 30 ml of PFA was used per animal. After extraction, brains were  
 1329 immersed in PFA 4% for an additional 2 hours, at 4C, and sliced on the same day as the perfusion.  
 1330 Parasagittal slices (100  $\mu$ m) containing S1 were prepared at 4°C using a vibratome (Leica  
 1331 VT1200S). Slices were kept at 4°C in a PBS-Azide 0.4% solution. In order to amplify the  
 1332 endogenous PSD95-mVenus fluorescence signal brain sections were immunostained with an anti-  
 1333 GFP Nanobody coupled to ATTO 647N (GBA647N-100, ChromoTek). For that brain sections  
 1334 were washed for 10 minutes in Phosphate Buffer (PB) 0.1M, followed by three 10 min incubation  
 1335 in Tris Buffer Saline (TBS). Sections were subsequently incubated for 1 hour in a blocking solution  
 1336 composed of 10% Normal Goat Serum (NGS), 0.2% triton 100-X in TBS. Directly after the  
 1337 blocking solution, brain sections were incubated for 2 hours in a solution of 2% NGS, 0.2% triton  
 1338 100-X, GFP-Booster ATTO647N (GBA647N-100, ChromoTek) (1:2000) in TBS. Sections were  
 1339 then washed (3 X 10 minutes) in TBS and rinsed in PB 0.1M and mounted on microscope slides  
 1340 using ProLongGlass Antifade Mountant (ProLong Glass, Invitrogen P36982). Fluorescence  
 1341 images were acquired at least 3 days after mounting to allow mounting medium to cure. Brain slices  
 1342 were imaged with a Stimulated Emission Depletion (STED) fluorescence microscope (expert line  
 1343 - Abberior Instruments) using an Olympus 100X/1.4 NA oil objective lens and 775 nm STED  
 1344 Laser line. Excitation laser lines were at 561nm and at 640nm respectively for TdTomato and Atto  
 1345 647N. STED Images were analyzed using Fiji.58.

1346 **Surgery for in vivo 2P imaging**

1347 Mice were initially anesthetized with Isoflurane. Induction of anaesthesia was performed at 4%  
1348 isoflurane, Iso-Vet, and an air flow of 250ml/min. Mice were then placed in a stereotactic device  
1349 (Kopf) kept at 37 °C using a regulated heating blanket and a thermal probe and maintained under  
1350 anaesthesia using 1-2% Isoflurane and an air flow of 250 ml/min until the end of the surgery. For  
1351 calcium imaging, cranial window implantation and AAV viral stereotaxic injection was performed  
1352 in the same surgical procedure. In order to prevent brain swelling and post-operative pain, anti-  
1353 inflammatory (Dexamethasone, 2 µg/g Dexadreson, MSD) and analgesic drugs (Buprenorphine,  
1354 0.1 mg/kg Buprecare) were injected subcutaneously. After shaving the scalp, the skin was cleaned  
1355 by wiping multiple times with an antiseptic solution and 70% alcohol. After injecting a local  
1356 anaesthetic under the scalp (lidocaine, 10mg/kg - Xylovet or Laocaine) a section of the skin was  
1357 removed using surgical scissors. The periosteum was then carefully removed and the skull was  
1358 scrapped using a dental drill, in order to remove all residues. The surface of the skull was  
1359 subsequently thoroughly cleaned and dried using cotton swabs and a physiological saline solution.  
1360 Using the stereotactic device, the center of the cranial window was marked and a circular piece of  
1361 bone was removed using a 3 mm biopsy punch (LCH-PUK-30 Kai Medical). This step of the  
1362 surgical procedure was considered critical since damage to the dura would result in rapidly  
1363 opacifying cranial windows. Once the brain was exposed, the AAV viral vectors carrying the  
1364 genetically encoded calcium indicators (GCaMP6s) were stereotactically injected. For calcium  
1365 imaging of SST-INs, AAV9 Syn-Flex-GCaMP6s-WPRE-SV40 (2.5x10<sup>13</sup> AddGene-100845) was  
1366 used respectively. Using a 10uL Gastight syringe 1701N (Hamilton), 200 nL of virus were injected  
1367 in the visual cortex (from bregma: 3.0 RC; 2.4 ML) at a rate of 1 nL/s followed by a 5 minutes  
1368 waiting period to prevent backflow. A round of 3mm glass coverslip (CS-3R Warner Instruments)  
1369 was then placed over the craniotomy and glued in place using dental cement (Superbond). A  
1370 stainless-steel head post (Luigs & Neumann) was then attached to the skull also using dental  
1371 cement. For 3 days following the cranial windows surgery, mice were injected once a day with a  
1372 mix of anti-inflammatory (Dexamethasone, 2 µg/g Dexadreson, MSD) and analgesic drugs  
1373 (Buprenorphine, 0.1 mg/kg Buprecare). One week after the surgery, animals were transferred into  
1374 an inverted light-dark cycle housing. Two weeks after surgery, mice were first habituated to the  
1375 experimenter by handling and the following days, mice were head-fixed in the acquisition setup  
1376 for increasing periods of time (5 – 45 minutes). The habituation of animals to the experimental  
1377 setup lasted at least 5 days with a minimum of 5 sessions. At the end of the habituation period  
1378 animals spontaneously transitioned between rest and running periods in the circular treadmill.  
1379 Animals were imaged between Zeitgeber time 12 and 24. Imaging sessions lasted between 30 to  
1380 60 min and animals were imaged between 3-5 days. The location of the injection site in V1 was  
1381 confirmed with intrinsic imaging experiments (69) as performed in our previous work (70).

1382

1383 **In vivo 2P Ca<sup>2+</sup> imaging**

1384 In vivo two-photon calcium imaging was performed with an Ultima IV two-photon laser-scanning  
1385 microscope system (Bruker), using a 20X, 1.0 N.A. water immersion objective (Olympus) with  
1386 the femtosecond laser (MaiTai DeepSee, Spectra Physics) tuned to 920 nm for imaging of cells  
1387 expressing GCaMP6s. Fluorescence light was separated from the excitation path through a long  
1388 pass dichroic (660dxxr; Chroma, USA), split into green and red channels with a second long pass  
1389 dichroic (575dxxr; Chroma, USA), and cleaned up with band pass filters (hq525/70 and hq607/45;  
1390 Chroma, USA). Fluorescence was detected using both proximal epifluorescence photomultiplier  
1391 tubes (gallium arsenide phosphide, H7422PA-40 SEL, Hamamatsu). Time-series movies of

1392 neuronal populations expressing GCaMP6s were acquired at the frame rate of 30Hz ( $512 \times 512$   
1393 pixels field of view;  $1.13 \mu\text{m}/\text{pixel}$ ). During the recording periods animals were free to run on a  
1394 circular treadmill covered with a soft foam. This foam softened the movement of the animals and  
1395 provided better traction (this was important since mice adapted more rapidly to the experimental  
1396 setup and movements were more fluid during the recordings).  
1397

### 1398 **Visual stimulation**

1399 Visual stimuli were presented at 60Hz on a Dell-2020 LED screen and synchronized to imaging  
1400 and behavioral data via a photodiode (placed on the top right of the screen) that recorded the timing  
1401 of each stimulus frame. Visual stimulation was controlled via the Psychopy software (71), version  
1402 2021.1.1. Full-field static gratings were presented for 2s (interleaved with 4s interstim intervals)  
1403 with 2 contrast levels (half-contrast and full contrast), 8 different orientations evenly spaced from  
1404  $0^\circ$  to  $157.5^\circ$  and a spatial frequency of  $0.04$  cycle/degree.  
1405 For two-photon experiments using a resonant scanner, the power source of the LED backlight of  
1406 the monitor was synchronized to the resonant scanner turnaround points (when data were not  
1407 acquired) to minimize light leak from the monitor (72).  
1408

### 1409 **Reduced model of dendritic integration**

1410 Adapting the work of (48), we studied dendritic integration by implementing cable theory in a  
1411 simplified model of the dendritic arborization. The overall dendritic structure is schematized in  
1412 **Fig. 11**, the morphology is made of 4 generations of branches with length  $50\mu\text{m}$ . At the end of  
1413 each generation  $b$ , each branch divides into two branches of a smaller diameter  $D_{b+1}=T \cdot D_b$  where  
1414  $T$  is the tapering factor. The root diameter ( $D_1$ ) was set to  $1.0\mu\text{m}$  to match our proximal  
1415 measurements (**Fig. S3**) and tapering factor was set to  $T=0.7$  so that the diameter reached  
1416  $D_3=0.5\mu\text{m}$  in the  $[100,150]\mu\text{m}$  of our distal measurements (**Fig. S3**). An isopotential spherical  
1417 compartment of radius  $15\mu\text{m}$  was added to model the soma. The membrane leak conductance was  
1418 adjusted to  $g_L=2.5\text{pS}/\mu\text{m}^2$  to match the  $\sim 100\text{M}\Omega$  input resistance for PV+ cells while the other  
1419 cable properties were fixed to classical values with the membrane capacitance set to  $c_m=1\mu\text{F}/\text{cm}^2$   
1420 and the intracellular resistivity to  $R_i=150\Omega.\text{cm}$ . Synaptic integration was tested by adding synaptic  
1421 conductance events with an instantaneous rise of amplitude  $1\text{nS}$  and an exponential decay of  
1422  $\tau=5\text{ms}$ . The reversal potential of the associated synaptic current was set to  $E_{\text{rev}}=0\text{mV}$ . This  
1423 morphology and electrical properties were implemented in the brian2 simulator (73) and simulated  
1424 with a time step  $dt=0.025\text{ms}$  and with a spatial discretization of  $n=20$  steps per branch.  
1425

### 1426 **Numerical simulations of morphologically-detailed PV- and SST-IN models**

1427 The morphologically-detailed models were implemented within the NEURON simulation  
1428 environment (74), version 8.2.3. From the Electron-Microscopy dataset of (45), we took the  
1429 detailed morphological reconstructions of the Basket (cell-ID: 864691135396580129) and  
1430 Martinotti (cell-ID: 864691135571546917) example cells shown in **Fig. 3G** for the PV-IN and  
1431 SST-IN models respectively. To focus on dendritic integration and for numerical simulation  
1432 efficiency, axonal compartments were removed from the reconstructions and only the somatic and  
1433 dendritic compartments were simulated. The passive properties of the models were constrained  
1434 from experimental measurements, see **Table S1**. **Fig 4-D** relies on passive properties only. To  
1435 investigate the spiking output properties following dendritic integration, we next added a minimal  
1436 set of active currents in **Fig 4E-G** and **Fig. 5A-D** for each model (**Table S2**). The action potential  
1437 currents were made of a fast voltage- dependent sodium channel (*gnafin*) and a delayed rectifier

1438 potassium channel (*gkdrin*) with densities adjusted to match the rheobase of experimentally  
1439 recorded cells (**Table S2**). For the PV-IN model, we also added A-type potassium channels (**Table**  
1440 **S2**) as this was shown to strongly participate in dendritic integration in PV-INs (*11*). For SST-INs,  
1441 we also added M-type potassium channels in the soma (**Table S2**) as those shape the regular-  
1442 spiking response characterizing SST-INs spiking profile. Action potentials were detected as a  
1443 positive crossing of the 0mV threshold. For each morphologically-detailed model, we manually  
1444 defined  $n=6$  dendritic branches that corresponded to a unique straightforward path starting near  
1445 the soma ( $<30\mu\text{m}$ ) and ending in a distal branch termination ( $>200\mu\text{m}$ ). Branches were insured not  
1446 to overlap with each other. Synapses were inserted on the morphology according to their locations  
1447 on the dendritic tree on the Electron-Microscopy dataset. Per dendritic branch, a “uniform”  
1448 surrogate of synaptic distribution was also created by randomly picking locations from a uniform  
1449 distribution of distance to the soma along the branch (see inset of **Fig. 4A**). In **Fig. 4E-G** and **Fig.**  
1450 **5A-D**, synapses were randomly split into 80% excitatory and 20% inhibitory synapses.  
1451 Synaptic currents from AMPA, GABA and NMDA receptors were taken from (*61*) and inserted  
1452 in the IN models (see properties in **Table S3**). For simplicity, NMDAR currents were only included  
1453 in the SST-IN model and not in the PV-IN model (**Table S3**, see also **Fig. 1G**). For the SST-IN  
1454 model in absence of NMDAR (condition “no NMDA, AMPA+”, see **Fig. 4E-G**), the AMPA  
1455 weight was increased by 50% to match the peak amplitude of PSPs at -60mV.  
1456 Background activity in **Fig. 4E-G** was achieved by stimulating each synapse with an independent  
1457 homogeneous Poisson point process. Rates of Poisson processes were adjusted to bring the neurons  
1458 in the -60mV range. For the PV-IN model, this was achieved  $F_{\text{inh}}=2.25\text{mHz}$  per synapses and  
1459  $F_{\text{exc}}=3\text{mHz}$  per synapse. For the SST-IN model, this was achieved with  $F_{\text{inh}}=8\text{mHz}$  per synapses  
1460 and  $F_{\text{exc}}=1\text{mHz}$  per synapse. In **Fig. 4F**, spiking response curves were fitted to the function:

$$1461 \quad F(n_{\text{syn}}) = A \cdot \text{Erf}\left((n_{\text{syn}} - n_{\text{syn}}^{1/2})/\sigma\right) \quad (8)$$

1462 where *Erf* is the error function (fitting with the method=*Nelder-Mead*” from the  
1463 *scipy.optimize.minimize* function).

1464 In **Fig. 5A-D**, the synapses were now driven by inhomogeneous Poisson processes, i.e. where they  
1465 were controlled by time-varying “input” rates (shown in grey in **Fig. 5A,B**). In **Fig. 5A**, the  
1466 excitatory and inhibitory rates are driven by step functions. In **Fig. 5B**, the rates are driven by an  
1467 Ornstein-Uhlenbeck stochastic process (i.e. temporally-correlated Gaussian fluctuations) of time-  
1468 constant  $\tau=50\text{ms}$  with mean  $\mu=2$  and variance  $\sigma^2=0.75^2$  multiplied by a scaling factor. This scaling  
1469 factor for synaptic activity rates (in mHz/synapse, see scale bars in **Fig. 5B**) was adjusted so that  
1470 the output firing rate reached  $20\pm 1\text{Hz}$  in all models considered (“PV”, “SST”, “SST:noNMDA,  
1471 AMPA+”). The output rate function was then obtained by binning spikes from  $n=50$  trials (“psth:  
1472 per-stimulus time histogram”) and smoothing the resulting trace with a Gaussian filter of width  
1473 10ms. The cross-correlation functions were fitted to Lorentzian function  $CC(\tau) = A \cdot$   
1474  $\frac{1}{1+(\tau-c)^2/\sigma^2} + B$  (constrained fitting with the method=*L-BFGS-B*” from the  
1475 *scipy.optimize.minimize* function) where  $\sigma$  is the width parameter that provides an estimate of the  
1476 temporal extent of the correlations to the input signal (**Fig. 5C**).

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## 1483 **Data analysis**

### 1484 **Synapse Distribution Analysis**

1485 We analyzed synaptic distributions on IN dendrites in a publicly-available 1mm<sup>3</sup> reconstruction of  
1486 the mouse visual cortex from a serial section transmission Electron Microscopy volume (44), i.e.  
1487 a reconstruction with synaptic resolution. To specifically analyze INs, we also benefited from the  
1488 manual proofread annotations of morphologically-identified INs found in (45) available at  
1489 <https://zenodo.org/record/7641781#collapseTwo>. As a proxy for PV- and SST-INs, we considered  
1490 the most numerous morphological type in each of those molecular classes, i.e. Basket and  
1491 Martinotti cells respectively. From Ref. (45), n=59 Basket cells and n=41 Martinotti cells were  
1492 available. For each cell, we benefited from a mesh reconstruction of its whole morphology as well  
1493 as the set of synapses targeting this cell (“pre”) or targeted by this cell (“post”) with their  
1494 localization in the volume (45). In **Fig. 3G,H**, we show such mesh reconstruction with the “pre”  
1495 synapses (note the presence of both the axonal and dendritic trees). As a first step, we converted  
1496 the mesh reconstructions of each cells into a “skeleton”, i.e. a set of segments with diameters, using  
1497 the package MeshParty (<https://meshparty.readthedocs.io/>, version 1.16.4) as this enables to easily  
1498 measure quantities such as “path length” along the dendritic tree (44). Next, we splitted axonal and  
1499 somato-dendritic compartments based on the density of “pre” and “post” synapses per  
1500 compartment (“pre” is high in dendrites/soma, “post” is high in axons) using the function  
1501 *meshwork.algorithms.split\_axon\_by\_annotation* from MeshParty. The few synapses (<1%)  
1502 located on the axonal compartments were excluded from further analysis. We next focused only  
1503 on the somato-dendritic compartments only. To analyze synapses along the dendritic path (**Fig.**  
1504 **3I,J**), the somato-dendritic skeleton of each cell was divided into a set of “dendritic cover paths”  
1505 covering the full dendritic tree (without any overlap). On all segments of this unique “dendritic  
1506 cover path”, we could compute the “path distance to soma” and count the number of synapses in  
1507 the segment to report either the linear density of synapses (**Fig. 3I**) or the absolute count (**Fig. 3J**)  
1508 as a function of the distance to soma.

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### 1510 **Visually-evoked spiking dynamics of PV- and SST-INs during natural movies**

1511 To analyze the temporal dynamics of the spiking activity in PV- and SST-INs, we re-analyzed the  
1512 publicly-available *Visual Coding* dataset from Ref. (50) of Neuropixels recordings with visual  
1513 stimulation and phototagging of INs. We considered the sessions in both the  
1514 “brain\_observatory1.1” and the “functional\_connectivity” datasets performed on Pvalb-IRES-  
1515 Cre;Ai32 (N=8 session) and Sst-IRES-Cre;Ai32 mice (N=12 sessions). We first restricted the  
1516 analysis to spiking units in the visual cortex (i.e. annotated as belonging to one of the visual areas:  
1517 “VISp”, “VISal”, “VISam”, “VISl”, “VISli”, “VISmma”, “VISmmp”, “VISp”, “VISpm”, “VISr”,  
1518 see (50)). We then splitted single units into positive and negative units with respect to the  
1519 molecular marker for INs. To this purpose, we analyzed the phototagging protocol (specifically  
1520 the 10ms light pulse stimulation) and computed the firing in the pre-stimulus [-10,0]ms and post-  
1521 stimulus [0,10]ms window. Units were defined as positive if their post-stimulus firing was above  
1522 20Hz and twice larger than the pre-stimulus firing (see “phototagging” insets in **Fig. 5F**). Using  
1523 this criterion, we found n=146 PV-positive units over N=6 sessions (n=24±14 units/session, two  
1524 sessions in Pvalb-IRES-Cre;Ai32 mice were excluded from the analysis because they had less than  
1525 2 positive units) and n=97 SST-positive units over N=12 sessions (n=8±6 units/session). To  
1526 estimate local cortical activity, we considered a random subset of n=100 negative units in each  
1527 session (out of the n=259±41 negative units/session in Pvalb-IRES-Cre;Ai32 mice and the  
1528 n=308±108 negative units/session in Sst-IRES-Cre;Ai32 mice). We next analyzed the spiking

1529 dynamics during the two natural movies that were presented in all sessions: “natural\_movie\_one”  
1530 and “natural\_movie\_three”. For each movie, we concatenated the spike times of the different units  
1531 and different movie presentations to build an average time-varying rate for a specific IN population  
1532 (see **Fig. 5F**). This time-varying rate was smoothed by a Gaussian kernel of width 10ms. We did  
1533 this either by pooling all units of all sessions for a single stimulus (shown in **Fig. 5G**, resulting in  
1534 a stimulus-evoked dynamics) or on a per-session basis (shown in **Fig. 5F**) to be able to analyze  
1535 properties across sessions (**Fig. 5H**). As in the model (**Fig. 5C**), the single-session cross-correlation  
1536 functions were fitted to a Lorentzian curve to estimate the temporal extent of correlations (**Fig.**  
1537 **5H**).

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### 1539 **Analysis of fluorescence signals**

1540 Two-photon imaging recordings of calcium activity were preprocessed with the **suite2p** software  
1541 (75) to perform the registration and the extraction of calcium signals. Identification of SST+ cells  
1542 were performed using the **cellpose** algorithm (76) on the time-averaged image of the field of view.  
1543 Raw fluorescence traces were corrected for neuropil contamination by subtracting Suite2p-  
1544 neuropil traces using a fixed scaling coefficient of 0.7 (75). To be able to compare activity across  
1545 cells and mouse lines, fluorescence signals were normalized. To do this we used the  $\Delta F/F_0$  method,  
1546 calculated using the following formula:  $(F-F_0)/F_0$ , where  $F$  is the fluorescence and  $F_0$  is the time-  
1547 varying baseline fluorescence evaluated as the lowest 5th percentile with a 2min sliding window.  
1548 After neuropil subtraction, calcium signals were deconvolved using the deconvolution algorithm  
1549 **oasis** from ref. (77) with settings from ref. (75) a (76) kernel time constant of 1.3s. Visually  
1550 responsive cells were defined as cells displaying a significant increase (Anova test,  $p < 0.05$ ) over  
1551 the  $n=10$  stimulus repetitions between the intervals  $[-1,0]$ s and  $[1,2]$ s preceding and following  
1552 stimulus presentation respectively.

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### 1554 **PSD-95 Venus puncta detection**

1555 PSD-95 puncta quantification was performed in dendrites simultaneously labelled with td-  
1556 Tomato). Td-Tomato fluorescence allowed effective dendrite tracing in order to estimate PSD-95  
1557 puncta density relative to soma distance. For a individual dendritic branches, image acquisitions  
1558 were performed proximally (less than 40um away from the soma) and distally (approximately  
1559 100um away from the soma). To avoid errors while defining distal and proximal dendritic  
1560 segments, we only imaged dendrites that did not cross other dendritic branches in the fields of  
1561 view. In addition, PSD95 quantification was performed in single images and not in z-stacks. Image  
1562 acquisition was performed for both td-tomato (excitation peak at 555nm) and ATTO647N for  
1563 mVenus (excitation peak at 647nm). Images were subsequently analyzed using Fiji software using  
1564 the Analyze Particles plugin. PSD-95 mVenus fluorescence images were first filtered using Fast  
1565 *Fourier transforms* using the Fiji FFT BandPass Filter function. Large structures (low frequency)  
1566 were filtered down to 60 pixels, small structures (high frequency) were filtered up to 2 pixels. The  
1567 images were thresholded using a minimum intensity threshold of the mean plus three times the  
1568 standard deviation of the background. Puncta detection was conducted using the Analyze Particles  
1569 plugin in Fiji, considering puncta with areas larger than 0.01 square micron. No maximal size limit  
1570 was defined. Puncta density was calculated by dividing the number of puncta counts by the length  
1571 of the analyzed dendritic segment. The length of the dendritic segment was defined on the td-  
1572 tomato fluorescence channel.

1573 **Statistics**

1574 We used Wilcoxon rank-sum tests for independent group comparisons, Wilcoxon signed-rank tests  
1575 for paired tests and Student's t-tests for a single group analysis. No statistical methods were used  
1576 to pre-determine sample sizes, but our sample sizes were similar to those used in previous  
1577 publications. Allocation into experimental groups was not randomized. Data collection and  
1578 analysis were not performed blind to the experimental conditions.

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1580 **Data availability**

1581 The code for the data analysis (EM dataset, Neuropixels dataset, Imaging dataset) and numerical  
1582 simulations (simplified morphological model, detailed biophysical and morphological models) of  
1583 this study is publicly available at the following link: <https://github.com/yzerlaut/pv-sst-dendrites>.

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