



HAL
open science

Developmental emergence of two-stage nonlinear synaptic integration in cerebellar interneurons

Célia Biane, Florian Ruckerl, Therese Abrahamsson, Cécile Saint-Clément, Jean Mariani, Ryuichi Shigemoto, David Digregorio, Rachel M Sherrard, Laurence Cathala

► **To cite this version:**

Célia Biane, Florian Ruckerl, Therese Abrahamsson, Cécile Saint-Clément, Jean Mariani, et al.. Developmental emergence of two-stage nonlinear synaptic integration in cerebellar interneurons. 2021. pasteur-03718292v1

HAL Id: pasteur-03718292

<https://hal.sorbonne-universite.fr/pasteur-03718292v1>

Preprint submitted on 14 Jan 2021 (v1), last revised 8 Jul 2022 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

Developmental emergence of two-stage nonlinear synaptic integration in cerebellar interneurons

Abbreviated title: **Development of neuronal computation in interneurons**

Célia Biane ¹, Florian Ruckerl ³, Therese Abrahamsson ³, Cécile Saint-Cloment ³, Jean Mariani ¹, Ryuichi Shigemoto ⁴, David A. DiGregorio ³ Rachel M. Sherrard ¹ and Laurence Cathala ^{1,2*}

¹ Sorbonne Université et CNRS UMR 8256, Adaptation Biologique et Vieillessement 9 Quai St Bernard, 75005 Paris, France

² Paris Brain Institute, CNRS UMR 7225 - Inserm U1127 – Sorbonne Université Groupe Hospitalier Pitié Salpêtrière 47 Boulevard de l'Hôpital 75013 Paris France

³ Department of Neuroscience, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France; CNRS URA 21821

⁴ Institute of Science and Technology Austria, Am Campus 1, 3400 Klosterneuburg, Austria

*Corresponding author: Laurence Cathala (Laurence.cathala@sorbonne-universite.fr)

Celia Biane : celiabiane1@gmail.com

Florian Ruckerl : florian.ruckerl@pasteur.fr

Therese Abrahamsson: therese.abrahamsson@gmail.com

Cécile Saint-Cloment : cecile.saint-cloment@pasteur.fr

David DiGregorio : david.digregorio@pasteur.fr

Ryuichi Shigemoto: ryuichi.shigemoto@ist.ac.at

Rachel Sherrard : rachel.sherrard@sorbonne-universite.fr

Jean Mariani : jean.mariani@sorbonne-universite.fr

33 **Abstract**

34

35 Synaptic transmission, connectivity, and dendritic morphology mature in parallel during brain
36 development and are often disrupted in neurodevelopmental disorders. Yet how these changes
37 influence the neuronal computations necessary for normal brain function are not well
38 understood. To identify cellular mechanisms underlying the maturation of synaptic
39 integration in interneurons, we combined patch-clamp recordings of excitatory inputs in
40 cerebellar stellate cells (SCs), 3D-reconstruction of SC morphology with excitatory synapse
41 location, and biophysical modeling. We found that, during development, synaptic strength
42 was homogeneously reduced along the somato-dendritic axis, but that dendritic integration
43 was always sublinear. However, dendritic branching increased without changes in synapse
44 density, leading to a substantial gain in distal inputs. Thus, changes in synapse distribution,
45 rather than dendrite cable properties, are the dominant mechanism underlying the maturation
46 of neuronal computation. These mechanisms favor the emergence of a spatially
47 compartmentalized two-stage integration model promoting location-dependent integration
48 within dendritic subunits.

49

50

51 **Introduction**

52

53 Dendritic integration of spatio-temporal synaptic activity is fundamental to neuronal
54 computation, which shapes the transformation of input activity into output spiking (Silver,
55 2010). In particular, the cable properties of dendritic trees generate isolated electrical
56 compartments that produce non-linear integration of synaptic potentials. These compartments
57 increase the computational power of single neurons (Caze et al., 2013; Poirazi and Mel,
58 2001) and are a prominent feature of human neurons (Beaulieu-Laroche et al., 2018; Gidon et
59 al., 2020). Dendritic morphology and ion channel expression are developmentally regulated,
60 but how they contribute to the maturation of neuronal computations throughout post-natal
61 circuit formation and refinement is less well known. The observation of alterations in
62 dendritic morphology, synaptic connectivity, density, and function in several
63 neurodevelopmental disorders (Marín, 2016; Penzes et al., 2011) indicates that both
64 appropriate neuronal wiring *and* the maturation of a neuron's integrative properties are
65 necessary to develop fully functional neuronal networks (Pelkey et al., 2015).

66

67 The type and number of computations that a neuron can perform depend on the diversity of
68 the mathematical operations used to combine synaptic inputs within dendritic trees. These
69 can be sublinear, linear, or supralinear (Branco and Häusser, 2011; Caze et al., 2013; Poirazi
70 and Mel, 2001; Tran-Van-Minh et al., 2015; Vervaeke et al., 2012). Nonlinear dendritic
71 operations depend on (1) dendritic architecture and associated membrane properties
72 (Abrahamsson et al., 2012; Hu et al., 2010; Katz et al., 2009; Larkum et al., 2009; Magee,
73 1999, 2000; Nevian et al., 2007; Rall, 1967), (2) spatial localization, density, and properties
74 of synapses across the dendritic arbor (Grillo et al., 2018; Larkum et al., 2009; Losonczy et
75 al., 2008; Losonczy and Magee, 2006; Menon et al., 2013; Schiller et al., 2000; Williams and
76 Stuart, 2002) and (3) the spatiotemporal synaptic activity pattern (Bloss et al., 2018; Grillo et
77 al., 2018; McBride et al., 2008; Scholl et al., 2017; Xu et al., 2012). All these factors change
78 during neuronal circuit maturation through cell-autonomous or activity-dependent processes
79 (Sigler et al., 2017) (Katz and Shatz, 1996). Indeed the maturation of neuronal excitability
80 and morphology (Cathala et al., 2003; Cline, 2016; McCormick and Prince, 1987; Zhang,
81 2004) is associated with restriction of neuronal connectivity to subcellular compartments
82 (Ango et al., 2004), activity-dependent synaptic rearrangement (Chen and Regehr, 2000;
83 Cline, 2016; Kwon and Sabatini, 2011; Li et al., 2011) and the maturation of excitatory
84 (Cathala et al., 2003; Hestrin, 1992; Koike-Tani et al., 2005; Lawrence and Trussell, 2000;
85 Taschenberger and von Gersdorff, 2000) and inhibitory synaptic inputs (Ben-Ari, 2002;
86 Sanes, 1993; Tia et al., 1996). Despite this knowledge, how developmental changes in
87 cellular parameters dictate dendritic operations and their associated neuronal computations,
88 remains largely unexplored.

89

90 Interneurons are fundamental to normal circuit function throughout development. They
91 contribute to the developmental regulation of critical periods (Hensch et al., 1998; Gu et al.,
92 2017), are important for establishing direction selectivity in the retina (Wei et al., 2011), and
93 their dysfunction is associated with neurodevelopment disorders (Akerman and Cline, 2007;
94 Le Magueresse, 2013; Marin, 2016). Parvalbumin-positive (PV+) GABAergic interneurons
95 are found in the neocortex, hippocampus, and cerebellum, and all share anatomical and
96 functional features (Hu et al., 2014). These inhibitory interneurons provide precise temporal
97 control of the excitatory drive onto principal neurons (Mittmann et al., 2005; Pouille and
98 Scanziani, 2001). Cerebellar stellate cells (SCs) are PV+ and receive excitatory inputs from
99 granule cells, and in turn modulate the excitability and firing precision of the cerebellar
100 output neurons, Purkinje cells (Arlt and Häusser, 2020; Häusser and Clark, 1997; Mittmann
101 et al., 2005). The thin SC dendrites (~0.4 μm diameter) filter synaptic potentials as they
102 propagate to the soma and confer sublinear summation of synaptic input (Abrahamsson et al.,
103 2012; Tran-Van-Minh et al., 2015). Nevertheless, the mechanisms underlying the maturation
104 of these dendritic operations and neuronal computation of interneurons has not been
105 explored.

106

107 Here we study in detail the maturation of the synaptic and integrative properties of SCs in the
108 cerebellar cortex. We combined patch-clamp recording with fluorescence-guided electrical
109 stimulation, fluorescence and electron microscopy 3D reconstructions, and numerical
110 simulations, to examine synapse strength and spatial distribution. Unlike unitary inputs in
111 other neuron types, we found that adult SCs had smaller and slower miniature excitatory
112 postsynaptic currents (mEPSCs) than those observed in immature SCs. This could be
113 explained by enhanced electrotonic filtering since immature SCs are thought to be
114 electrotonically compact (Carter and Regehr, 2002; Llano and Gerschenfeld, 1993). We
115 found, however, that their dendrites are as thin as in adult SCs and also capable of robust
116 electrotonic filtering and sublinear summation of synaptic inputs. Using a novel fluorescence
117 synaptic tagging approach we found a significantly larger contribution of distal dendritic
118 synapses in adult SCs, due to a substantial increase in dendritic branching combined with
119 constant synapse density. Multicompartment biophysical modeling confirmed that
120 developmental changes in synapse distribution could reproduce the developmental reduction
121 and slowing of recorded mEPSCs as well as the increased sublinear integration observed in
122 adult SCs. Our findings provide evidence that SCs implement different neuronal
123 computations throughout development: A predominant global summation model in immature
124 SCs shifts to sublinear dendritic integration in adult SCs, favoring the developmental
125 emergence of the two-layer integration model. This work provides a mechanistic description
126 of the maturation of neuronal computation resulting from both functional and anatomical

127 changes in synaptic transmission and integration. Our findings and approach also provide a
128 framework for interpreting the functional implications of alterations in dendritic morphology
129 and connectivity on information processing within neural circuits during disease.

130

131

132 **Results**

133

134 **AMPA-mediated mEPSCs become smaller and slower during development**

135 The strength and time course of synaptic transmission is fundamental to information
136 processing within neural networks since they influence the efficacy and temporal precision of
137 the transformation of synaptic inputs into neuronal outputs. Excitatory synaptic inputs trigger
138 postsynaptic conductance changes due to the opening of neurotransmitter-gated receptors
139 which are activated upon transmitter release. These conductance changes are locally
140 integrated within dendrites into excitatory postsynaptic potentials (EPSPs), which then
141 propagate to the soma where they contribute to somatic voltage. The strength and time course
142 of synaptic conductances are known to change during development (Cathala et al., 2003;
143 Chen and Regehr, 2000; Koike-Tani et al., 2005) and can affect dendritic integration, which
144 in turn may alter neuronal computation (Tran-Van-Minh et al., 2015).

145

146 To identify factors that shape the post-natal development of SC integrative properties, we
147 first compared excitatory postsynaptic currents (EPSCs) recorded in acute brain slices, either
148 from immature SCs soon after they reach their final position in the outer layer of the
149 cerebellar cortex (postnatal days 13 to 17), or from adult SCs (post-natal ages 35 to 57).
150 Granule cell afferent (parallel fibers, PFs) synapses release glutamate from only one synaptic
151 vesicle, despite the presence of multiple release sites per synaptic contact (Foster et al.,
152 2005). We, therefore, examined these physiologically relevant “quantal synaptic events”
153 using somatic recordings of spontaneously occurring AMPA receptor (AMPA)-mediated
154 miniature EPSCs (mEPSCs) in the presence of TTX to block spontaneous presynaptic
155 activity. mEPSCs arise from the release of a single neurotransmitter vesicle and occur
156 randomly at all synapses converging onto a single neuron. Therefore, mEPSCs can provide an
157 unbiased assessment of the effective distribution of synaptic strengths throughout the entire
158 somato-dendritic compartment.

159

160 We found that AMPAR-mediated mEPSCs occurred with a similar frequency at both ages
161 (1.37 ± 0.27 vs. 1.14 ± 0.13 Hz, $P > 0.05$), but mEPSCs were significantly smaller and slower
162 in the adult (Figures 1A-1C). In immature SCs the average mEPSC amplitude was 48 ± 7 pA,
163 with 10-90% rise and decay times (τ_{decay} , see Methods) of 0.16 ± 0.01 ms and 0.68 ± 0.06 ms
164 respectively. In contrast, mEPSCs from adult SCs were smaller 24 ± 2 pA ($P < 0.05$), with
165 slower rising (0.24 ± 0.02 ms; $P < 0.05$) and decaying kinetics ($\tau_{\text{decay}} = 1.31 \pm 0.14$ ms;
166 $P < 0.05$). The mEPSC amplitudes are consistent with those from previous studies describing
167 large miniature events (Llano and Gerschenfeld, 1993) capable of influencing immature SC
168 firing (Carter and Regehr, 2002). Nevertheless, we observed that mEPSCs continue to mature
169 past the 3rd post-natal week, becoming smaller and slower.

170

171 Previous studies have described developmental alterations in the glutamate content of
172 synaptic vesicles (Yamashita, 2003) and synaptic structure (Cathala et al., 2005), both of
173 which can modulate the neurotransmitter concentration in the synaptic cleft. To test whether
174 the reduced amplitude and slower time-course could be due to alteration in *effective*
175 amplitude and time-course of glutamate concentration ([Glut]) seen by synaptic AMPARs, we
176 recorded mEPSCs in the presence of a rapidly dissociating, low-affinity competitive AMPAR
177 antagonist, γ DGG (Diamond and Jahr, 1997; Liu et al., 1999). Application of a submaximal
178 concentration of γ DGG (1 mM) reduced mEPSC peak amplitude (Figure 1D, paired $P < 0.05$)
179 similarly at both ages ($44.42 \pm 4.36\%$, $n = 7$ in the immature vs. $42.38 \pm 3.69\%$, $n = 9$ in the
180 adult, $P > 0.05$; Figure 1E), with no apparent effect on mEPSC kinetics (Figure 1F, paired
181 $P > 0.05$). This result suggests that the decreased amplitude and slowing of mEPSCs is
182 unlikely due to a change in the synaptic [Glut]. We, therefore, explored whether post-synaptic
183 mechanisms, such as the acquisition of dendritic electrotonic filtering, (which exists in adult
184 SCs (Abrahamsson et al., 2012) and/or lower synaptic conductance (i.e., the number of
185 activated synaptic AMPARs), could explain the changes in mEPSC during maturation.

186

187 **Dendritic morphology supports electrotonic filtering in both immature and adult SCs**

188 Dendrites of adult SCs exhibit electrotonic cable filtering, which reduces the amplitude of
189 synaptic responses and slows their time course as they propagate to the soma (Abrahamsson
190 et al., 2012), thus modifying mEPSCs recorded at the soma. We considered whether the
191 developmental difference in mEPSC amplitude and kinetics was due to the development of
192 electrotonic filtering. To test this hypothesis, we estimated the dendrite diameter of immature
193 SCs, because the small diameter ($< 0.5 \mu\text{m}$) and rapid synaptic conductances of adult SC
194 dendrites underlie their cable filtering properties (Abrahamsson et al., 2012). The dendritic
195 diameter was estimated from the full-width at half-maximum (FWHM) of the fluorescence
196 profile perpendicular to the dendrite from confocal images of live SCs aged P13 to P17 filled
197 with Alexa 488 (Figure 2A). Diameters ranged from $0.26 \mu\text{m}$ to $0.93 \mu\text{m}$ with a mean of 0.47
198 $\pm 0.01 \mu\text{m}$ ($n = 93$ dendritic segments of 18 neurons; Figure 2B), which is close to the
199 average adult value of $0.41 \pm 0.02 \mu\text{m}$ (range 0.24 to $0.9 \mu\text{m}$, $n = 78$ dendrites; data from
200 Abrahamsson et al., 2012; $P < 0.05$).

201

202 To understand the potential functional influence of such small diameters, we calculated the
203 dendritic space constant (see Methods), i.e. the distance along a cable over which a steady-
204 state membrane voltage decreases by $1/e$. Using the estimated $0.47 \mu\text{m}$ dendritic diameter,
205 membrane resistance (R_m) of $20,000 \text{ Ohm.cm}^2$ (matching that experimentally measured from
206 immature SCs membrane time constant τ_m of $19 \pm 2.2 \text{ ms}$, $n = 16$) and an internal resistivity
207 R_i ranging from 100 to 200 Ohm.cm , we calculated the steady-state dendritic space constant

208 (λ) to be between 343 to 485 μm , which is 3-5 fold longer than the actual dendritic length.
209 This confirms that, for steady-state membrane voltages, immature SCs are indeed electrically
210 compact, as previously suggested (Carter and Regehr, 2002). However, the frequency-
211 dependent space constant (λ_r ; assuming that rapid mEPSCs are well-approximated by a 1 kHz
212 sine wave), was calculated to be between 46 to 60 μm (for R_i of 100 - 200 $\text{Ohm}\cdot\text{cm}$). These
213 values are less than dendrite lengths and suggest that, like in adult SCs (Abrahamsson et al.,
214 2012), somatic recording of EPSC originating in dendrites may be smaller and slowed due to
215 electrotonic cable filtering.

216
217 We confirmed these frequency-dependent estimations using a multi-compartmental
218 biophysical model to simulate the somatic response to quantal synaptic release throughout the
219 somato-dendritic compartment. We first used an idealized SC dendritic morphology
220 (Abrahamsson et al., 2012) and then fully reconstructed immature SC dendritic trees. For the
221 idealized immature SC morphology (Figure 2C), we used an 8 μm soma diameter ($8.07 \pm$
222 $0.23 \mu\text{m}$, estimated from confocal images of 31 immature SC somata) and a $0.47 \mu\text{m}$ dendritic
223 diameter (see mean value from Figure 2B), an R_m of 20,000 $\text{Ohm}\cdot\text{cm}^2$ and an R_i of 100 - 200
224 $\text{Ohm}\cdot\text{cm}$. The simulated synaptic conductance g_{syn} amplitude and time course were adjusted
225 to match quantal EPSCs generated by a single vesicle (qEPSC; see experimental approach
226 below and Figure 3) recorded following activation of somatic synapses. Simulated qEPSCs
227 were large and fast for synapses located at the soma (magenta trace, Figure 2C) and 72%
228 smaller and 200% slower when synapses were located on the dendrites (grey trace; for a
229 synapse at 47 μm on a dendrite with 3 branch points and an intermediate R_i of 150 $\text{Ohm}\cdot\text{cm}$).
230 This decrease in amplitude was associated with a large increase in the local synaptic
231 depolarization (green trace, Figure 2C) that would substantially reduce the local driving force
232 during synaptic transmission onto dendrites, causing a sublinear read-out of the underlying
233 conductance, similar to that observed in adult SCs (Abrahamsson et al., 2012).

234
235 We obtained similar results with morphologically accurate passive biophysical models
236 derived from 3D reconstructed SCs. SCs were patch-loaded with the fluorescence indicator
237 Alexa 594, imaged with two-photon laser scanning microscopy (2PLSM; Figure 2D), and
238 then reconstructed with *NeuronStudio* (Rodriguez et al., 2008). The 3D reconstruction was
239 then imported into the simulation environment, *Neuron*, with the membrane properties
240 indicated above. Activation of a synaptic contact at 60 μm from the soma on any dendrite of
241 the reconstructed immature P16 SC, produced a simulated qEPSC that was consistently
242 smaller and slower (grey traces) than the one produced following the activation of a somatic
243 synapse (magenta trace; Figure 2E). Similarly, activation of synaptic inputs along a dendrite,
244 at increasing distance from the soma, produced soma-recorded qEPSCs that become smaller
245 and slower with distance (Figure 2F), similar to those in reconstructed adult SCs (Figure 2G,

246 2H) and idealized SC models (Abrahamsson et al., 2012). These simulations suggest that, like
247 their adult counterparts, the morphometric characteristics of immature SCs should also
248 produce significant cable filtering of both the amplitude and time course of EPSCs.

249

250 **Synaptic events are electrotonically filtered in immature SCs**

251 To confirm modeling predictions, we next explored whether dendrite-evoked quantal events
252 in immature SCs show evidence of cable filtering. Taking advantage of the orthogonal
253 projection of parallel fibers (PFs), we used parasagittal cerebellar slices to stimulate specific
254 PF beams that are synaptically connected to well-defined regions of an Alexa 594 loaded SC
255 by placing an extracellular electrode either above the soma or close to the distal part of an
256 isolated dendrite branch (Figure 3A and 3B). We recorded evoked qEPSCs using whole-cell
257 voltage clamp of the SC soma. This approach allows precise control of the location of the
258 activated synapses, in contrast with mEPSCs that can arise from unknown synapse locations
259 anywhere along the somato-dendritic axes. Dendritic filtering could then be examined by
260 measuring the amplitude and time course (response width at half-peak, half-width) of these
261 synaptic events, typically used to estimate cable filtering (Rall, 1967). To isolate qEPSCs,
262 PFs were stimulated in low release probability conditions (EPSC success rate of less than
263 10%; 0.5 mM extracellular $[Ca^{2+}]$ and 5 mM $[Mg^{2+}]$). In these conditions, the average EPSC
264 generated from all successful trials is a good approximation of the quantal current amplitude
265 and time course (Silver et al., 2003). When stimulating somatic synapses, qEPSCs recorded
266 at the soma had a mean peak amplitude of 62 ± 3 pA, a 10-90 % rise time of 0.14 ± 0.004 ms,
267 and a half-width of 0.60 ± 0.02 ms ($n = 25$; Figure 3C, 3D). In contrast, stimulation of
268 dendritic synapses produced somatically recorded qEPSCs that were significantly smaller
269 (mean amplitude: 46 ± 3 pA) and slower (10-90 % rise time of 0.24 ± 0.012 ms and a half-
270 width of 0.88 ± 0.04 ms; $n = 18$; all $P < 0.05$, Figure 3C, 3D). These results are consistent with
271 cable filtering of EPSCs as they propagate along dendrites in immature SCs.

272

273 The decreased amplitude of qEPSCs evoked in the dendrite could also be due to lower
274 AMPAR content of dendritic synapses. As AMPAR density in SC synapses is constant
275 (Masugi-Tokita et al., 2007) we used post-synaptic density (PSD) size as a proxy for the
276 number of AMPARs. We measured PSD area of somatic and dendritic synapses from 3D
277 electron microscopy (EM) reconstruction of immature SCs. We reconstructed the soma and
278 the dendritic tree of two SCs (P14 and P17) loaded with Alexa 594 and biocytin (Figure 3E).
279 Immunogold labeling of biocytin made possible the identification of PSDs (Figure 3F) and
280 measurement of their distance from the soma. PSD area was constant along somatodendritic
281 axes (Figure 3G) ruling out synaptic scaling as a mechanism for the reduction in qEPSCs
282 after dendritic stimulation. Thus the difference in qEPSC amplitude and time-course observed
283 between somatic and dendritic synapses in immature SCs is likely due to cable filtering.

284

285 **Developmental changes in synaptic conductance amplitude, but not time course**

286 Because cable filtering of synaptic responses is present in both immature and adult SCs, we
287 next explored whether differences in mEPSC amplitude between the two ages were due to
288 maturation of quantal synaptic conductance (i.e. number of synaptic AMPARs). To avoid the
289 effects of cable filtering, we measured qEPSCs only at somatic synapses. In immature SCs,
290 somatic qEPSCs were 41% larger than those in adult SCs (62.3 ± 3.3 pA, $n = 25$ vs. 44 ± 2.4
291 pA, $n = 12$, Abrahamsson et al., 2012), $p < 0.05$, Figure 4A, 4B), but with no difference in the
292 half-width (0.60 ± 0.02 ms, $n = 25$ vs. 0.59 ± 0.06 ms, $n = 12$, $P > 0.05$, Figure 4B). These
293 results suggest that more AMPARs are activated by quantal release of glutamate at immature
294 somatic synapses. Similarly, the 10-90 % rise time was slower in immature SCs compared to
295 adult (0.14 ± 0.02 ms, $n = 25$ vs. 0.12 ± 0.004 , $n = 12$, $P < 0.05$; Figure 4B), indicating larger
296 immature synapses (Cathala et al., 2005). To confirm the reduction in synaptic AMPARs
297 during maturation, we compared somatic PSD areas between immature and adult SCs. In
298 immature SCs, mean somatic PSD size was 0.039 ± 0.002 μm^2 ($n = 83$ synapses; Figure 4C),
299 which is 39% larger than that of adult SCs (0.028 ± 0.0015 μm^2 , $n = 97$, $P < 0.05$; data
300 obtained by the same method from Abrahamsson et al., 2012). The developmental reduction
301 in PSD size is similar to the amplitude reduction of recorded somatic qEPSC, and thus will
302 contribute to the developmental reduction in mEPSC. However, fewer synaptic AMPARs
303 cannot explain the observed change in mEPSC time course.

304

305 **Dendritic distribution of excitatory synaptic inputs changes during maturation**

306 In addition to cable filtering of synaptic currents, a neuron's somatic response to dendritic
307 input is also affected by synapse distribution within its dendritic tree, which may not be
308 uniform – as demonstrated for starburst amacrine interneurons (Vlasits et al., 2016) and CA1
309 pyramidal neurons (Katz et al., 2009; Magee and Cook, 2000). We hypothesized that changes
310 in synapse distribution could underlie the slowing of mean mEPSCs time-course observed
311 adult SCs. In support of this hypothesis, immature SC mEPSC rise and decay kinetics are
312 similar to those of somatic qEPSCs (compare Figure 1 vs. Figure 4), whereas adult SC
313 mEPSC kinetics are closer to those of dendritic qEPSCs (Figure 1 vs. Figure 3). Specifically,
314 in immature SC the mean mEPSC decay is similar to the qEPSC decay from somatic
315 synapses but significantly different from those of dendritic synapses, suggesting that synaptic
316 responses from distal synapses do not participate significantly to the mean mEPSC at this
317 developmental stage. In contrast, the adult mEPSC decay is close to the dendritic qEPSC
318 decay, but significantly different from that of somatic synapses. These results are consistent
319 with mEPSCs in adult SCs arising more often from more distal synapses.

320 To test this hypothesis, we examined the distribution of excitatory synaptic inputs along the
321 somato-dendritic compartment in immature and adult SCs. Since SC dendrites lack spines,

322 we used transgenic mice conditionally expressing Venus-tagged PSD95 to label putative
323 excitatory synapses. We then mapped Venus-tagged PSD95 puncta associated with the somata
324 and dendritic trees of Alexa 594-filled immature and adult SCs (Figure 5A-D), defining
325 puncta located within 200 nm of the dendritic surface (Figure 5E) as excitatory synapses
326 targeting the dendrite (Figure 5F; see Methods). Venus-tagged PSD95 puncta within the soma
327 and dendritic tree of 9 immature and 8 adult 3D-reconstructed SCs (Figure 5G), showed ~ 80
328 % more puncta on adult SCs (582 ± 48 puncta vs. 324 ± 21 in immature SC, $p < 0.05$).
329 Synapse distribution was assessed by counting the number of PSD95 puncta within 10 μ m
330 segments at increasing distance from the soma. In immature SCs, ~ 80 % are within 35 μ m of
331 the soma, in contrast to only ~ 40% this close to the soma in adult SCs (Figure 6A, 6B).
332 However, the ratio of detected puncta (Figure 6B) to dendritic segments (Figure 6C) shows
333 that puncta density remained constant across the dendritic tree at both ages (Figure 6D).
334 Thus, the larger number of puncta located further from the soma in adult SCs is not due to
335 increased puncta density with distance, but a bigger dendritic field (Figure 6E) and many
336 more distal dendritic branches (Sholl analysis, Figure 6F). Taken together, these data
337 demonstrate that increased dendritic complexity during SC maturation is responsible for a
338 prominent shift toward distal synapses in adult SCs. Therefore, if mEPSCs are generated
339 from a homogeneous probability of release across all synapses, then this bias towards distal
340 synapses in adult SC will generate quantal responses that experience stronger cable filtering.

341

342 **Change in synapse distribution underlies developmental slowing of mEPSC**

343 We next examined whether differences in synapse distribution could account quantitatively
344 for the observed changes in mEPSC amplitude and time course. We performed numerical
345 simulations using reconstructed immature and adult SCs (Figure 2D and 2G) and a quantal
346 synaptic conductance (g_{syn}) that reproduced measured immature and adult qEPSCs induced at
347 somatic synapses (Figure 4). We simulated qEPSCs evoked by synaptic activation at the
348 soma and at 10 μ m intervals along the somato-dendritic axes (Figure 7A). Assuming that
349 mEPSCs are generated randomly with an equal probability at all synapses, we generated a
350 simulated *mean mEPSC* by summing the qEPSC at each distance (EPSC_d), each weighted by
351 its relative frequency according to the synapse distribution (Figure 7B). EPSC_d s arising from
352 distal locations in the adult were larger and contributed relatively more to the simulated *mean*
353 mEPSC waveform (Figure 7B). The resulting *mean mEPSC* was smaller in adult SCs than in
354 immature SCs (26.0 ± 0.6 pA, $n = 9$ dendrites vs. 52.2 ± 0.4 pA, $n = 7$ for R_i 150) and its
355 time-course had slower rise (10-90 % rise time = 0.24 ± 0.05 vs. 0.17 ± 0.01 ms) and decay
356 (half-width = 1.11 ± 0.04 vs. 0.77 ± 0.01 ms; for all $P < 0.05$). Moreover, the simulated *mean*
357 mEPSCs lay within one standard deviation of measured experimental mEPSC values (Figure
358 7C, 7D). Thus by implementing the experimentally observed synapse distributions in our
359 simulations, we could accurately reproduce the experimental mean mEPSCs. These results

360 demonstrate that developmental increases in dendritic branching complexity, provided that
361 synapse density is homogeneous, can account for the changes in mEPSC kinetics (Figure 1).

362

363 **Influence of synapse distribution on dendritic integration of multiple synaptic inputs**

364 We previously showed in adult SCs that the activation of dendritic synapses conveys
365 sublinear integration as compared to the soma (Abrahamsson et al., 2012) due to the large
366 local input resistance of thin dendrites resulting in synaptic depolarizations that reduced
367 synaptic current driving force (Bloomfield et al., 1987; Rall, 1967). This also produced a
368 distance-dependent decrease in short-term plasticity (STP). We examined whether dendritic
369 integration in immature SCs is also sublinear by comparing STP following dendritic and
370 somatic synapse activation. PFs targeting SC somata and dendrites were stimulated using a
371 pair of extracellular voltage pulses with an interval of 20 ms. The paired-pulse ratio (PPR; the
372 ratio of the amplitudes of the second vs. the first EPSC) was 2.1 ± 0.1 for somatic synapses (n
373 = 10) and decreased to 1.8 ± 0.1 for distal dendritic synapses ($n = 15$; $P < 0.05$; Figure 8A),
374 consistent with sublinear integration. These results were reproduced by numerical simulations
375 of evoked EPSCs or EPSPs in the idealized passive SC model (with a synaptic g_{syn} matching
376 the recorded EPSC evoked at the soma and a 2.25 conductance ratio). These findings show
377 that immature SC dendrites also display an STP gradient, suggesting that they are capable of
378 sublinear integration.

379

380 To address this possibility, we used biophysical modeling of subthreshold synaptic input-
381 output relationships (I/O) that has been shown to accurately reproduce experimental sublinear
382 I/Os recorded after neurotransmitter photo-uncaging (Abrahamsson et al., 2012). Evoked
383 EPSPs were simulated (sim eEPSP) in response to increasing synaptic conductance (g_{syn}),
384 equivalent to one to 20 quanta in order to encompass sparse and clustered activation of
385 parallel fibers (Wilms and Häusser, 2015) at the soma and at 10 μm intervals along the
386 reconstructed dendrites of the immature SC. (Figure 8B). I/O plots showed that sim eEPSPs
387 in the soma and dendrites were less than the linear sum of eEPSPs (dashed line). This
388 sublinear summation was apparent for dendritic eEPSPs generated from synaptic
389 conductances equivalent to 1 quantum for dendritic synapses and became more pronounced
390 for distal synapses (Figure 8C). The sublinearity increased with increasing number of
391 simultaneously activated quanta. These simulations show that immature dendrites
392 demonstrate sublinear summation, supporting experimental difference between somatic and
393 dendritic STP (Figure 8A).

394

395 To estimate the impact of development changes in synapse distribution on the maturation of
396 SC computations, we compared simulated subthreshold I/Os between immature and adult
397 SCs with their respective age-dependant g_{syn} (Figure 8C). For immature SCs we examined

398 subthreshold I/Os when activating synapses at 15 μm along reconstructed dendrites (Figure
399 8C, green), a distance with the highest relative number of synaptic contacts (Figure 7B), and
400 compared to I/Os generated from synapse activation at 45 μm in adult SCs (distance with the
401 largest relative number of synapses). Sublinearity was quantified by normalizing sim eEPSPs
402 to an sim eEPSP evoked by injecting a g_{syn} of 0.1 quanta, a conductance to which the voltage
403 is linearly related. Because large g_{syn} can generate sublinear integration at the soma (Figure
404 8C), we estimated the dendrite-specific sublinearity for each g_{syn} by taking the ratio between
405 the relative sim eEPSPs amplitude (normalized to quanta) at a given distance and the
406 normalized sim eEPSP amplitude for somatic synapses. The final estimate of dendritic
407 sublinearity was then defined as one minus this ratio (Figure 8D). While both immature and
408 adult SCs exhibited sublinear integration, dendritic sublinearity was larger in adult SCs for all
409 synaptic strengths, supporting an increased difference between the two layers of integration
410 (i.e. soma versus dendrite). The smaller difference in sublinearity between soma and dendrite
411 in the immature SC resulted from both fewer distal synapses and the larger g_{syn} . Thus the
412 developmental increase in dendritic field complexity and decreased synaptic strength together
413 contribute to the establishment of a two-stage integration model and provide a cellular
414 substrate for a developmental increase in computational power of SCs.
415

416 **Discussion**

417
418 Dendritic integration of synaptic inputs is a critical component of neuronal information
419 processing, but little is known about how it matures during neuronal network formation and
420 maturation. We took advantage of the late development of the cerebellar cortex to
421 characterize developmental changes in synaptic and dendritic properties. By combining
422 patch-clamp recording of cerebellar SC interneurons, 3D-reconstructions of their aspiny
423 dendrites, along with the identification of excitatory synapse locations, and numerical
424 simulations, we showed for the first time how the maturation of synapse distribution within
425 interneurons combines with changes in synaptic strength and increased dendritic branching to
426 shape the development of neuronal computation. This maturation process favors the
427 emergence of a compartmentalized two-stage integrator model, which extends the repertoire
428 of transformations of synaptic inputs into neuronal output in adult SCs. These results
429 highlight the importance of characterizing not only dendritic morphology, but also synapse
430 placement and synaptic strength, in order to correctly infer a neuron's computational rules.
431

432 **Implications of developmental alterations of dendritic morphology**

433 We showed that soon after SC integration into the cerebellar molecular layer microcircuit,
434 their dendrites are nearly the same diameter as adult SCs (Abrahamsson et al., 2012),
435 suggesting a similar capacity for cable filtering of synaptic responses. Since previous studies

436 suggested that SCs were electronically compact (Carter and Regehr, 2002; Llano and
437 Gerschenfeld, 1993), the observation that mEPSCs from adult SCs were slower led us to
438 consider changes in cable filtering as the underlying mechanism for the developmental
439 change in mEPSC kinetics. However, we showed, both experimentally and by modeling, that
440 dendrite-evoked qEPSCs from immature SCs have similar dendritic integration properties as
441 adult SCs (Abrahamsson et al., 2012): (a) dendrite-evoked qEPSCs are smaller and slower
442 than those evoked at the soma (Figure 3), consistent with cable filtering; (b) STP differed
443 between dendrite and somatic stimulation (Figure 8); and (c) subthreshold I/Os were
444 sublinear (Figure 8). Thus the basic electrotonic machinery to filter synaptic responses is
445 already present as soon as SC precursors reach their final location in the outer third of the
446 molecular layer. However, the maturational difference between mEPSCs, which presumably
447 reflect synaptic responses from the entire dendritic tree, suggested that another factor must be
448 contributing to the difference in apparent electrotonic filtering.

449

450 Previous studies have shown that synapses are not uniformly distributed along dendrites,
451 allowing pyramidal neuron dendrites to operate as independent computational units (Katz et
452 al., 2009; Menon et al., 2013; Polsky et al., 2004), and retinal starburst amacrine interneurons
453 to compute motion direction (Vlasits et al., 2016). We considered the possibility that the
454 distribution of synapse locations within the dendritic tree was altered during SC maturation.
455 We found that synapses were uniformly distributed along the somato-dendritic axis with a
456 similar density at the two ages. However, adult SCs had more synapses located at further
457 electrotonic distances ($\sim 2/3$ vs. $1/3$ of synapses were more than $30 \mu\text{m}$ from the soma; Figure
458 6 and 7) due to increased dendritic branching. Thus, the distal-weighted synaptic distribution
459 in adult SCs favors inputs that experience stronger cable filtering. This was confirmed by
460 simulating a mean mEPSC at the two ages, that fully reproduce the mEPSC recorded
461 experimentally (Figure 1), by weighting simulated qEPSCs according to the relative number
462 of synapses at specific distances along the dendrites (Figure 7). Indeed, the large fraction of
463 distal synapses in adult SCs was sufficient to account for the observed developmental
464 difference in mEPSC amplitude and time-course.

465

466 **Synaptic conductance does not display distance-dependence synaptic scaling**

467 Voltage-clamp recordings of mEPSCs showed that their time course and amplitude are both
468 halved during development (Figure 1). This result contrasts with findings in other neurons
469 that show faster mEPSCs during maturation due to changes of AMPAR subunits, glutamate
470 vesicular concentration content, and/or synapse structure (Cathala et al., 2005; Yamashita et
471 al., 2003). Knowing that dendritic inputs could be electrotonically filtered, we took advantage
472 of the ability to selectively stimulate somatic synapses to isolate somatic qEPSCs for
473 comparison between the two ages. Evoked qEPSCs showed a developmental reduction in

474 amplitude (~ 40%), with no change in kinetics (Figure 4). This likely results from the smaller
475 adult PSD size (Figure 4), and hence a lower AMPAR number (Masugi-Tokita et al., 2007),
476 rather than a developmental reduction in the peak glutamate concentration at the postsynaptic
477 AMPARs (Figure 1). Therefore individual synaptic inputs are less likely to influence adult
478 SC neuronal output. Moreover, since PSD area is constant along the somato-dendritic axis
479 (Figure 3), the observed developmental reduction in synaptic conductance can be
480 extrapolated to the whole dendritic tree. Thus SCs do not exhibit synaptic conductance
481 scaling mechanisms to offset dendritic filtering, such has been described for pyramidal
482 neurons (Katz et al., 2009; Magee and Cook, 2000; Menon et al., 2013; Nicholson et al.,
483 2006), resulting in a strong dependence of somatic voltage responses on synapse location
484 within the dendritic arbor.

485

486 **Developmental changes in computational rules**

487 Our findings highlight the critical importance of understanding both the structural and
488 functional mechanisms underlying developmental refinement of synaptic integration that
489 drives a neuron's computational properties and, the emergence of mature microcircuit
490 function. While a defining feature of immature SCs is the high propensity of quantal EPSPs
491 to generate spikes (Carter and Regehr, 2002), the observed developmental decrease in
492 synaptic conductance (Figure 4) and increased filtering of mEPSCs (Figures 2 and 3) will
493 tend to reduce the influence of single synaptic inputs on somatic voltage in adult SCs,
494 increasing their dynamic range of subthreshold integration. Although dendrites in immature
495 and adult SCs exhibit similar electrotonic filtering, the distal bias in synapse location
496 promotes sublinear subthreshold dendritic integration in adult SCs. Unlike pyramidal
497 neurons, where synapse strength and density are scaled to normalize the contribution of
498 individual inputs to neuronal output (Katz et al., 2009; Magee and Cook, 2000; Menon et al.,
499 2013), the spatially uniform distribution of synapse strength and density in SCs do not
500 compensate the electrotonic filtering effects of the dendrites or the increased number of distal
501 synapses due to branching.

502

503 These properties of quantal synaptic responses, together with the larger difference in
504 sublinearity between soma and dendrites (Figure 8D), will favor the emergence of a spatially
505 compartmentalized two-stage integration model in adult SCs, thereby promoting location-
506 dependent integration within dendritic subunits (Polsky et al., 2004) and enhanced neuronal
507 computations (Caze et al., 2013). In immature SCs, the repertoire of computations is more
508 similar to a simple single-stage integration model where large and fast synaptic potentials
509 will promote reliable and precise EPSP-spike coupling (Cathala et al., 2003; Fricker and
510 Miles, 2001; Hu et al., 2010), which may be critical for driving the functional maturation of
511 the local microcircuit (Akgül et al., 2020). In contrast, synaptic integration and summation in

512 adult SCs can obey different rules depending on synapse location within the dendritic tree
513 enabling to discriminate a larger number of spatial patterns of synaptic activation (Tran-Van-
514 Minh et al., 2015) and therefore favor spatially sparse synaptic representations (Abrahamsson
515 et al., 2012; Caze et al., 2013) that might be essential for the development of enhanced
516 pattern separation by Purkinje cells (Cayco-Gajic et al., 2017). Since a recent theoretical
517 study showed that sublinear integration is also a property of hippocampal fast-spiking
518 interneurons (Tzilivaki et al., 2019) that influence memory storage, it will also be important
519 to determine if these interneurons exhibit a similar maturation of their neuronal computation.

520

521 **Implications for neurodevelopmental and neurological disorders**

522 The increasing complexity of dendritic arbors, accompanying changes in synaptic
523 connectivity and function during development is not limited to the cerebellum. These
524 maturational processes are altered in neurodevelopmental disorders, such as mental
525 retardation (Kaufmann and Moser, 2000), autism spectrum disorders (Antoine et al., 2019;
526 Peng et al., 2016), or Rett Syndrome (Blackman et al., 2012; Ip et al., 2018), as well as in
527 neurodegenerative disease. Indeed, these developmental processes are particularly relevant
528 for interneurons since they play a pivotal role in the establishment of the correct
529 excitation/inhibition balance for normal circuit function. During development, inhibitory
530 interneurons are essential for defining critical periods (Gu et al., 2016; Hensch et al., 1998) or
531 direction selectivity in the retina (Vlasits et al., 2016; Wei et al., 2011), so that interneuron
532 dysfunction is associated with neurodevelopment disorders (Akerman and Cline, 2007; Le
533 Magueresse and Monyer, 2013; Marín, 2016). Our work demonstrates how developmental
534 changes in neuronal morphology, and synapse distribution and strength, combine to
535 determine the impact of synaptic inputs on neuronal output. Our findings provide a functional
536 template of how dendritic integration matures throughout development to enrich interneurons
537 with more complex neuronal computations, promoting location-dependent integration within
538 dendritic subunits.

539

540

541 **Materials and Methods**

542 **Slice preparation and Electrophysiology**

543 Acute cerebellar parasagittal slices (250 or 200 μm thick respectively) were prepared from
544 immature (postnatal day 14-17) and adult (P35-57) mice (F1 of BalbC and C57B6 or
545 C57BL/6J) as described previously (Abrahamsson et al., 2012). Briefly, mice were killed by
546 decapitation, the brains rapidly removed and placed in an ice-cold solution containing (in
547 mM): 2.5 KCl, 0.5 CaCl₂, 4 MgCl₂, 1.25 NaH₂PO₄, 24 NaHCO₃, 25 glucose, and 230
548 sucrose, bubbled with 95% O₂ and 5% CO₂. Slices were cut from the dissected cerebellar
549 vermis using a vibratome (Leica VT 1000S or VT1200S), incubated at 32°C for 30 minutes in
550 the following solution (in mM): 85 NaCl, 2.5 KCl, 0.5 CaCl₂, 4 MgCl₂, 1.25 NaH₂PO₄, 24
551 NaHCO₃, 25 glucose, and 75 sucrose and subsequently maintained at room temperature for
552 up to 8 hours in the recording solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl₂, 1
553 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, and 25 glucose. Unless otherwise noted, this solution
554 included during patch recordings 10 μM SR-95531, 10 μM D-AP5, 20 μM 7-chlorokynurenic
555 acid, and 0.3 μM strychnine, to block GABA_A, NMDA, and glycine receptors, respectively.

556

557 Whole-cell patch-clamp recordings were made from SCs located in the outer one-
558 third at molecular layer at temperatures ranging from 33 to 36°C using an Axopatch-200A or
559 a Multiclamp 700 amplifier (Axon Instruments, Foster City, Ca, USA) with fire-polished
560 thick-walled glass patch-electrodes (tip resistances of 6-8 M Ω) that were backfilled with a
561 solution containing (in mM): 117 K-MeSO₄, 40 HEPES, 6 NaOH, 5 EGTA, 1.78 CaCl₂, 4
562 MgCl₂, 1 QX-314-Cl, 0.3 NaGTP, 4 NaATP, and, when applied 0.03 Alexa 594, adjusted to
563 ~300 mOsm and pH 7.3. Series resistance and capacitance measures were determined directly
564 from the amplifier settings.

565

566 All EPSCs were recorded at -70 mV (not corrected for LJP ~ +6 mV) were filtered at
567 10 kHz, and digitized at 100 kHz using an analogue-to-digital converter (model NI USB
568 6259, National Instruments, Austin, TX, USA) and acquired with Nclamp (Rothman and
569 Silver, 2018) within the Igor Pro 6.2 environment, WaveMetrics). No series resistance
570 compensation was used. To evoke EPSC, parallel fibers were stimulated with a glass patch
571 electrode filled with external recording solution that was placed close to a fluorescently
572 labeled dendrite or close to the soma. 50 μs pulses between 5-55 V (Digitimer Ltd,
573 Letchworth Garden City, UK) were delivered as described previously (Abrahamsson et al.,
574 2012). Somatic and dendritic quantal EPSCs were obtained from experiments where [Ca²⁺]
575 was lowered to 0.5 mM while [Mg²⁺] was increased to 5 mM to obtain an evoked EPSC
576 success rate <10% known to produce a qEPSC with a <10% amplitude error (Silver, 2003).
577 Trials with a synaptic event could be clearly selected by eye. The stimulation artifact was
578 removed by subtracting from single success traces the average obtained for the traces with

579 failed synaptic transmission.

580

581 Current-clamp recordings were performed using a Multiclamp 700 amplifier. Patch electrodes
582 were coated with dental wax and series resistance was compensated by balancing the bridge
583 and compensating pipette capacitance. Current was injected to maintain the resting potential
584 near -70 mV. Data were filtered at 10 kHz, and digitized at 100 kHz.

585

586 D-AP5, 7-chlorokynurenic acid, γ DGG, QX-314 chloride, SR 95531 and Tetrodotoxin were
587 purchased from Ascent Scientific (<http://www.ascentscientific.com>). Alexa 594 was
588 purchased from Invitrogen (<https://www.thermofisher.com/invitrogen>). All other drugs and
589 chemicals were purchased from Sigma-Aldrich (<https://www.sigmaaldrich.com>).

590

591 **Multi-compartmental biophysical modeling**

592 Passive cable simulations of EPSC and EPSP propagation within idealized and reconstructed
593 SC models were performed using Neuron 7.1, 7.2 and 7.5 (Hines and Carnevale, 1997). The
594 idealized SC model had a soma diameter of 9 μm and three 90 μm long dendrites of 0.47 μm
595 diameter, with either 1, 3 or 5 branches. An immature (P16) and adult SC (P42) were
596 reconstructed in 3D with NeuronStudio (Rodriguez et al., 2008) from 2PLSM image of SC
597 patch loaded with 30 μM Alexa 594 in the pipette and imported in Neuron. Passive properties
598 were assumed uniform across the cell. Specific membrane capacitance (C_m) was set to 0.9
599 $\mu\text{F}/\text{cm}^2$. R_m was set to 20 000 Ωcm^2 to match the membrane time constant experimentally
600 estimated at 19 ± 2.2 ms for immature SCs ($n = 16$) and 17 ± 2.7 ms for adult SC ($n=10$). R_i
601 was set to 150 Ωcm to match the filtering of EPSC decay in the dendrites of mature SC
602 (Abrahamson et al., 2012) and allowed to vary from 100 to 200 Ωcm to sample a large range
603 of physiological R_i since its physiological value is not known. The AMPAR-mediated
604 conductance waveforms (gSyn) were set to match the amplitude and kinetics of experimental
605 somatic qEPSCs and evoked EPSCs. Experimental somatic PPR for EPSCs were reproduced
606 with a gSyn2/gSyn1 of 2.25.

607

608 **Electron microscopy and three-dimensional reconstructions**

609 Electron microscopy and three-dimensional (3D) reconstructions of two SCs from acute
610 slices (postnatal day 14 and 17) were performed as described previously (Abrahamsson et al.,
611 2012). Slices containing SCs whole-cell patched with a K-MeSO₃-based internal solution
612 containing biocytin (0.3%) and Alexa 594 (30 μM) were transferred to a fixative containing
613 paraformaldehyde (2.5%), glutaraldehyde (1.25%), and picric acid (0.2%) in phosphate buffer
614 (PB, 0.1 M, pH = 7.3), and fixed overnight at room temperature. After washing in PB, slices
615 were transferred to sucrose solutions (15% for 30 minutes, then stored in 30%) for
616 cryoprotection and frozen in liquid nitrogen, then subsequently thawed. The freeze-thaw

617 cycle was repeated twice, then followed by incubation with a 1.4nm gold-conjugated
618 streptavidin (Nanoprobe, 1:100 in Tris-buffered saline (TBS) and 0.03% Triton X100). After
619 washing in TBS and dH₂O, slices were treated with HQ silver enhancement kit (Nanoprobe)
620 for 5 minutes, fixed in 1% OsO₄ in PB for 30 minutes, and block stained with 1% uranyl
621 acetate for 40 minutes. After dehydration through a series of ethanol solutions (50, 70, 80, 90,
622 95, 99, and 100%) and propylene oxide twice for 10 minutes, slices were embedded into
623 Durcupan (Fluka) and flat embedded. The labeled SCs were trimmed and 300~400 serial
624 ultrathin sections were cut at 70 nm using ATUMtome (RMC Boeckeler). Serial sections
625 containing immunogold labeled profiles were imaged with a scanning electron microscope
626 (Merlin Compact, Zeiss) and Zeiss Atlas package at X 22,000 for whole-cell reconstruction
627 and at X 55,000 for synapses. For the area measurement of synapses on soma, serial sections
628 through three unlabeled neighbor SCs were also used to avoid potential turbulence due to the
629 patching. These sections were cut at 70 nm using an ultramicrotome (Leica EM UC7),
630 observed with a transmission electron microscope (Tecnai 12, FEI), and photographed at X
631 21,000. Asymmetrical synapses made by axon terminals onto SC somata and dendrites were
632 analyzed only if they were fully present within the serial sections. The PSD length of the
633 asymmetrical synaptic membrane specialization was measured on each ultrathin section, and
634 the PSD area was calculated by multiplying the summed synaptic length from each synapse
635 with the thickness (70 nm) of the ultrathin sections. The 3D reconstruction of the two SC
636 soma and parts of their dendritic trees was performed using the software Reconstruct (JC
637 Fiala). The distances from each synapse to the soma were measured along the dendrites in the
638 reconstructed volume.

639

640 **Transmitted light and fluorescence imaging**

641 SC somata in the outer one-third of the molecular layer were identified and whole-cell
642 patched using infrared Dodt contrast (Luigs and Neumann). Two-photon excitation or LED
643 illumination coupled with the Dodt contrast was used to visualize Alexa 594 filled SCs and
644 position extracellular stimulating electrodes along isolated dendrites of SCs fluorescence.
645 Two-photon excitation was performed with a pulsed Ti:Sapphire laser (MaiTai DeepSee,
646 Spectra Physics) tuned to 810 nm and images were acquired with an Ultima two-photon laser
647 scanning microscope system (Bruker) mounted on an Olympus BX61WI microscope
648 equipped with a 60x (1.1 NA) water-immersion objective. LED excitation (470 nm) was
649 performed with a CAIRN LED module (optoLED) and wide-field fluorescence images were
650 acquired with a CCD camera (QIclick, QImaging) mounted on an Olympus BX51
651 microscope equipped with a 60x (1 NA) water-immersion objective.

652

653 One-photon confocal laser scanning fluorescence microscopy was performed with an Ultima
654 scan head mounted on a Nikon EFN microscope. SCs were filled with 40 μ M Alexa 488.

655 Maximal intensity projections of confocal images were performed using a 100x 1.1 NA
656 Nikon dipping objective in 0.2 μm increments as described previously (Abrahamsson et al.,
657 2012). We used the full-width at half maximum (FWHM) of intensity line profiles on 1 μm
658 segments of dendrites, made perpendicular to dendritic length, as an approximation of the
659 dendritic diameter. This is likely to be an upper limit given the blurring effect of the PSF.

660

661 *2P imaging*: The two-photon scanning microscope (2PLSM, Ultima IV, Bruker) was
662 equipped with a Ti:Sapphire Laser (Chameleon II, Coherent Inc.) at 940nm (SC body, Alexa
663 594) and 810nm (venus-tagged PSD95 puncta) using a 60x water immersion objective
664 (LUMFL N, 1.10 NA, Olympus). The point spread function of the microscope was estimated
665 from the FWHM value of the x-,y- and z- intensity line profiles from 3D images of 200nm
666 yellow-green fluorescent latex beads (FluoSpheres, F8811, Thermo Fisher Scientific):
667 $\text{PSF}_{810x/y} = 325 \pm 27$ (SD) nm, $\text{PSF}_{810z} = 1178 \pm 121$ (SD) nm, and $\text{PSF}_{940x/y} = 390 \pm 23$ (SD),
668 $\text{PSF}_{940z} = 1412 \pm 141$ (SD) nm.

669

670 **Three-dimensional reconstructions and puncta detection from 2P images**

671 We examined the distribution of excitatory synaptic inputs along the somato-dendritic
672 compartment in SCs from a transgenic mouse line that conditionally expresses Venus-tagged
673 PSD95 under the control of the nitric oxide synthase 1 promoter (PSD95-Enabled (Fortin et
674 al., 2014) x Nos1 Cre (Kim et al., 2014)). We patch-loaded single SCs with the fluorescence
675 indicator Alexa 594, then performed live two-color 2PLSM to identify Venus-tagged PSD95
676 puncta associated with the labeled somata and dendritic trees. Z-stacks were acquired for
677 each wavelength with a z-step of 300 nm, a pixel size of 154 nm, and an image size of 512 x
678 512 pixels. To correct for a shift in the focal point for the different wavelengths and a
679 potential drift in x/y-directions, the individual stacks were registered to each other using as a
680 reference the dendritic structure imaged using Alexa 594 emission, which is primarily excited
681 at 810 nm, but weakly excited at 940 nm which allows to record both the puncta and the cell
682 body simultaneously. The registration was performed using the IMARIS *stitcher* tool.
683 Fluorescence emission was spectrally separated from laser excitation using a custom multi-
684 pass dichroic (zt 405/473-488/nir-trans; Chroma) and a short pass IR blocking filter
685 (ET680sp-2p8; Chroma). Venus and Alexa 594 fluorescence emission were spectrally
686 separated and detected using detection filter cubes consisting of a long-pass dichroic
687 (575dcxr; Chroma) and two bandpass filters (HQ525/70m-2p and HQ607/45m-2p,
688 respectively; Chroma). A multi-alkali (R3896, Hamamatsu, Japan) photomultiplier tubes was
689 used to detect Alexa 594 fluorescence and gallium arsenide phosphide tube (H7422PA-40
690 SEL, Hamamatsu) for the Venus channel. Proximal and substage detection were used to
691 increase signal to noise.

692 *Dendrite tracing:* The image analysis software IMARIS 9.5 (Bitplane) was used for dendritic
693 tracing and fluorescence puncta detection. Fluorescence images were filtered using a 3x3 px
694 median filter to remove noise. Image stacks were then further combined using the IMARIS
695 *stitcher* tool to create one contiguous file that permits tracing of the entire dendritic tree.
696 Dendrites, but not axons, were traced in IMARIS using the *filament* tool in a semi-automatic
697 mode using the *AutoPath* method and the options *AutoCenter* and *AutoDiameter* activated.
698 The soma center was chosen as the starting point with centripetally tracing along dendrites.
699 Dendritic lengths were estimated as the dendritic path distance from the center of the soma
700 and Sholl analysis was performed with IMARIS from all reconstructed SC.

701 PSD-95 Venus puncta detection: Fluorescence puncta were detected using the IMARIS spot
702 creation tool, using background fluorescence subtraction to compensate for different levels of
703 fluorescent intensity along the z-axis of the stack. The initial minimal spot search size was set
704 to 300x300x1100 nm, slightly smaller than the PSF at 810 nm. No further thresholds or
705 criteria were applied inside IMARIS. Parameters describing the fluorescent puncta, including
706 the intensity at the center, their spatial coordinates, and their diameters, were exported as
707 excel files via the Statistics tab and further analyzed using custom python scripts. In order to
708 separate the PSD95 puncta from false detection of noise, two threshold criteria were applied.
709 1) All spots with a diameter smaller than and equal to the PSF (300x300x1100nm) were
710 rejected. 2) Only spots with a peak intensity larger than the mean of the background intensity
711 plus three times its standard deviation of the background noise, were considered for
712 subsequent analysis. In combination, the thresholds ensure that the spots originated from
713 fluorescent puncta and not false positives generated from noise fluctuations. The background
714 intensity and its corresponding standard deviation was measured for each file in *Fiji*, by
715 selecting regions without puncta and using the *Measure* tool to calculate the mean and
716 standard deviation. In order to ensure consistent sampling of the background, mean and noise
717 estimates were made from at least ten different regions at different z positions in each stack,
718 corresponding to an area between 17-27 μm^2 (790 - 1210 pixels) and 10 - 40 μm^2 (430 -
719 1850 pixels), for the immature and adult SCs, respectively. This approach is limited by the
720 resolution of 2P fluorescence imaging to differentiate individual synapses within clusters and
721 thus may result in an underestimate of absolute synapse density, but allowed for an unbiased
722 estimate of synapse distributions at the two developmental stages.

723 Puncta located on somata were selected from the total pool of detected puncta (described
724 above) using the following criteria: 1) spots were associated with the soma if the peak
725 intensity at the position of the spot center in the Alexa 594 channel was larger than half the
726 maximum of the whole stack, 2) detected spot diameters were greater than the size PSF
727 (300x300x1100nm), 3) spot intensities were larger than the mean plus 3*SD of background
728 intensity of the PSD95-venus channel, measured from within the soma. Puncta from somata

729 that showed saturation in the Alexa 594 channel were not included in the analysis.

730 *Analysis of spot and dendrite distances:* The structure of the dendritic tree, as well as the
731 position of the puncta, were further analyzed using custom python scripts. The dendritic tree
732 was reconstructed with the center of the soma as its root using the python *NetworkX* package.
733 Fluorescence puncta were considered to arise from the labeled dendrite if they were located
734 within a maximal distance from the center of the dendrite. This distance was taken as the
735 dendritic radius, estimated from IMARIS, plus 200 nm (~HWHM of the PSF₈₁₀). The
736 estimation of local radius was made from IMARIS binary masks using a threshold of the
737 local contrast (*DiameterContrastThreshold*) set at three times the standard deviation above
738 the background fluorescence noise. The diameter is then calculated using the *Shortest*
739 *Distance from Distance Map* algorithm, which estimates the diameter as the distance from the
740 center of the dendrite to the closest part of the surface determined by the above threshold. The
741 average dendritic radius, using this approach, was found to be 0.66 ± 0.28 (SD) μm for adult,
742 and 0.72 ± 0.27 (SD) μm for immature mice. As this value was larger than that estimated
743 from single-photon confocal imaging, it was only used as a part of the criteria for assigning a
744 fluorescence puncta to a reconstructed dendrite. For each dendritic branch, the number of
745 PSD95 puncta and their distance to the soma surface were calculated. As the data points of
746 the dendrite structure obtained from IMARIS are not homogeneously spaced, the dendritic
747 structure was resampled in 100 nm intervals with the distance for each segment recalculated
748 with respect to the starting point of each corresponding branch from the soma surface
749 (estimated using the binary mask as for the dendrites). Histograms for the distribution of
750 PSD95 puncta and the number of dendritic segments at a given distance from the soma were
751 then generated in 10 μm bins and used to estimate the puncta density along the dendritic tree.
752 Cumulative plots were sampled at 1 μm intervals.

753

754 **Electrophysiology analysis**

755 Data analysis was performed using the Neuromatic analysis package (Rothman and Silver,
756 2018) written within the Igor Pro environment (WaveMetrics, Lake Oswego, OR, USA).
757 mEPSCs were detected with a threshold detection method and mEPSC population average is
758 calculated from the mean EPSC response calculated for each SC. All EPSC were baseline
759 subtracted using a 1 ms window before the stimulation artifact. Peak amplitudes were
760 measured as the difference between the baseline level immediately preceding the stimulation
761 artifact, and the mean amplitude over a 100 μs window centered on the peak of the response.
762 EPSC decay kinetics were assessed either as the width of the EPSC at the amplitude one-half
763 of the peak (half-width in ms) or as the weighted time constant of decay (τ_{decay}) calculated
764 from the integral of the current from the peak, according to:

765

766

767

768

769

770

$$t_{decay} = \frac{\int_0^{t_{\infty}} I(t) dt}{I_{peak}}$$

771 where t_{peak} is the time of the EPSC peak, t_{∞} is the time at which the current had returned to
772 the pre-event baseline, and I_{peak} the peak amplitude of the EPSC.

773

774 All data are expressed as average \pm SEM otherwise noted. Statistical tests were performed
775 using a nonparametric Wilcoxon-Mann-Whitney two-sample rank test routine for unpaired or
776 a Wilcoxon signed-rank test routine for paired comparisons. Kolmogorov–Smirnov test (KS
777 test) was used to compare cumulative distributions. Unless otherwise noted, unpaired tests
778 were used and considered significant at $P < 0.05$ (OriginPro, Northampton, MA, USA)

779

780 **Equation 1.** Length constant for an infinite cable.

781

$$\lambda_{DC} = \sqrt{\frac{dR_m}{4R_i}},$$

782

783

784 where d is the dendritic diameter and R_m and R_i are the specific resistance of the membrane
785 and internal resistivity, respectively.

786

787

788 **Equation 2.** Frequency-dependent length constant for an infinite cable.

789

$$\lambda_{AC} = \lambda_{DC} \sqrt{\frac{2}{1 + \sqrt{1 + (2\pi f \tau_m)^2}}},$$

790

791

792 where f is the frequency representing an AMPAR current and τ_m is the membrane time
793 constant.

794

795 When f is greater than 100 Hz, this can be simplified when f is greater than 100 Hz:

796

797

798

799

800

801

$$\lambda_{AC} \approx \sqrt{\frac{d}{4\pi f R_i C_m}}$$

802

803 **Acknowledgements:**

804 This study was supported by the Centre National de la Recherche Scientifique and the
805 Agence Nationale de la Recherche (ANR-13-BSV4-00166, to LC and DAD). TA was
806 supported by fellowships from the Fondation pour la Recherche Medicale and the Swedish
807 Research Council. We thank Dmitry Ershov from the Image Analysis Hub of the Institut
808 Pasteur, Elodie Le Monnier, Elena Hollergschwandtner, Vanessa Zheden and Corinne Nantet
809 for technical support and Haining Zhong for providing the Venus-tagged PSD95 mouse line.
810 We would like to thank Alberto Bacci, Ann Lohof and Nelson Rebola for comments on the
811 manuscript.

812

813 **Competing interests:**

814 The authors have no competing financial or non-financial interests.

815

816

817 **References**

818

819 Abrahamsson T, Cathala L, Matsui K, Shigemoto R, DiGregorio DA. 2012. Thin dendrites of
820 cerebellar interneurons confer sublinear synaptic integration and a gradient of short-term
821 plasticity. *Neuron* **73**:1159–72. doi:10.1016/j.neuron.2012.01.027

822 Akerman CJ, Cline HT. 2007. Refining the roles of GABAergic signaling during neural
823 circuit formation. *Trends Neurosci* **30**:382–389. doi:10.1016/j.tins.2007.06.002

824 Ango F, di Cristo G, Higashiyama H, Bennett V, Wu P, Huang ZJ. 2004. Ankyrin-based
825 subcellular gradient of neurofascin, an immunoglobulin family protein, directs
826 GABAergic innervation at purkinje axon initial segment. *Cell* **119**:257–272.
827 doi:10.1016/j.cell.2004.10.004

828 Antoine MW, Langberg T, Schnepel P, Feldman DE. 2019. Increased Excitation-Inhibition
829 Ratio Stabilizes Synapse and Circuit Excitability in Four Autism Mouse Models. *Neuron*
830 **101**:648-661.e4. doi:10.1016/j.neuron.2018.12.026

831 Arlt C, Häusser M. 2020. Microcircuit Rules Governing Impact of Single Interneurons on
832 Purkinje Cell Output In Vivo. *Cell Rep* **30**:3020-3035.e3.
833 doi:10.1016/j.celrep.2020.02.009

834 Ben-Ari Y. 2002. Excitatory actions of gaba during development: the nature of the nurture.
835 *Nat Rev Neurosci* **3**:728–739. doi:10.1038/nrn920

836 Blackman MP, Djukic B, Nelson SB, Turrigiano GG. 2012. A critical and cell-autonomous
837 role for MeCP2 in synaptic scaling up. *J Neurosci Off J Soc Neurosci* **32**:13529–13536.
838 doi:10.1523/JNEUROSCI.3077-12.2012

839 Bloomfield SA, Hamos JE, Sherman SM. 1987. Passive cable properties and morphological
840 correlates of neurones in the lateral geniculate nucleus of the cat. *J Physiol* **383**:653–692.
841 doi:10.1113/jphysiol.1987.sp016435

842 Bloss EB, Cembrowski MS, Karsh B, Colonell J, Fetter RD, Spruston N. 2018. Single
843 excitatory axons form clustered synapses onto CA1 pyramidal cell dendrites. *Nat*
844 *Neurosci* **21**:353–363. doi:10.1038/s41593-018-0084-6

845 Branco T, Häusser M. 2011. Synaptic integration gradients in single cortical pyramidal cell
846 dendrites. *Neuron* **69**:885–892. doi:10.1016/j.neuron.2011.02.006

847 Carter AG, Regehr WG. 2002. Quantal events shape cerebellar interneuron firing. *Nat*
848 *Neurosci* **5**:1309–18.

849 Cathala L, Brickley S, Cull-Candy S, Farrant M. 2003. Maturation of EPSCs and intrinsic
850 membrane properties enhances precision at a cerebellar synapse. *J Neurosci* **23**:6074–85.

851 Cathala L, Holderith NB, Nusser Z, DiGregorio DA, Cull-Candy SG. 2005. Changes in
852 synaptic structure underlie the developmental speeding of AMPA receptor-mediated
853 EPSCs. *Nat Neurosci* **8**:1310–8.

854 Cayco-Gajic NA, Clopath C, Silver RA. 2017. Sparse synaptic connectivity is required for
855 decorrelation and pattern separation in feedforward networks. *Nat Commun* **8**:1116.
856 doi:10.1038/s41467-017-01109-y

857 Caze RD, Humphries M, Gutkin B. 2013. Passive dendrites enable single neurons to compute
858 linearly non-separable functions. *PLoS Comput Biol* **9**:e1002867.
859 doi:10.1371/journal.pcbi.1002867

860 Chen C, Regehr WG. 2000. Developmental remodeling of the retinogeniculate synapse.
861 *Neuron* **28**:955–966. doi:10.1016/s0896-6273(00)00166-5

862 Cline HT. 2016. Dendrite development Dendrites. Oxford University press. pp. 77–94.

- 863 Diamond JS, Jahr CE. 1997. Transporters buffer synaptically released glutamate on a
864 submillisecond time scale. *J Neurosci Off J Soc Neurosci* **17**:4672–4687.
- 865 Fortin DA, Tillo SE, Yang G, Rah JC, Melander JB, Bai S, Soler-Cedeno O, Qin M,
866 Zemelman BV, Guo C, Mao T, Zhong H. 2014. Live imaging of endogenous PSD-95
867 using ENABLED: a conditional strategy to fluorescently label endogenous proteins. *J*
868 *Neurosci* **34**:16698–712. doi:10.1523/JNEUROSCI.3888-14.2014
- 869 Foster KA, Crowley JJ, Regehr WG. 2005. The influence of multivesicular release and
870 postsynaptic receptor saturation on transmission at granule cell to Purkinje cell synapses.
871 *J Neurosci Off J Soc Neurosci* **25**:11655–11665. doi:10.1523/JNEUROSCI.4029-05.2005
- 872 Fricker D, Miles R. 2001. Interneurons, spike timing, and perception. *Neuron* **32**:771–774.
873 doi:10.1016/s0896-6273(01)00528-1
- 874 Gidon A, Zolnik TA, Fidzinski P, Bolduan F, Papoutsi A, Poirazi P, Holtkamp M, Vida I,
875 Larkum ME. 2020. Dendritic action potentials and computation in human layer 2/3
876 cortical neurons. *Science* **367**:83–87. doi:10.1126/science.aax6239
- 877 Grillo FW, Neves G, Walker A, Vizcay-Barrena G, Fleck RA, Branco T, Burrone J. 2018. A
878 Distance-Dependent Distribution of Presynaptic Boutons Tunes Frequency-Dependent
879 Dendritic Integration. *Neuron* **99**:275–282.e3. doi:10.1016/j.neuron.2018.06.015
- 880 Gu Y, Tran T, Murase S, Borrell A, Kirkwood A, Quinlan EM. 2016. Neuregulin-Dependent
881 Regulation of Fast-Spiking Interneuron Excitability Controls the Timing of the Critical
882 Period. *J Neurosci Off J Soc Neurosci* **36**:10285–10295. doi:10.1523/JNEUROSCI.4242-
883 15.2016
- 884 Häusser M, Clark BA. 1997. Tonic synaptic inhibition modulates neuronal output pattern and
885 spatiotemporal synaptic integration. *Neuron* **19**:665–678. doi:10.1016/s0896-
886 6273(00)80379-7
- 887 Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF. 1998. Local
888 GABA circuit control of experience-dependent plasticity in developing visual cortex.
889 *Science* **282**:1504–1508. doi:10.1126/science.282.5393.1504
- 890 Hestrin S. 1992. Developmental regulation of NMDA receptor-mediated synaptic currents at
891 a central synapse. *Nature* **357**:686–689. doi:10.1038/357686a0
- 892 Hu H, Gan J, Jonas P. 2014. Interneurons. Fast-spiking, parvalbumin⁺ GABAergic
893 interneurons: from cellular design to microcircuit function. *Science* **345**:1255263.
894 doi:10.1126/science.1255263
- 895 Hu H, Martina M, Jonas P. 2010. Dendritic mechanisms underlying rapid synaptic activation
896 of fast-spiking hippocampal interneurons. *Science* **327**:52–58.
897 doi:10.1126/science.1177876
- 898 Ip JPK, Mellios N, Sur M. 2018. Rett syndrome: insights into genetic, molecular and circuit
899 mechanisms. *Nat Rev Neurosci* **19**:368–382. doi:10.1038/s41583-018-0006-3
- 900 Katz LC, Shatz CJ. 1996. Synaptic activity and the construction of cortical circuits. *Science*
901 **274**:1133–1138. doi:10.1126/science.274.5290.1133
- 902 Katz Y, Menon V, Nicholson DA, Geinisman Y, Kath WL, Spruston N. 2009. Synapse
903 distribution suggests a two-stage model of dendritic integration in CA1 pyramidal
904 neurons. *Neuron* **63**:171–177. doi:10.1016/j.neuron.2009.06.023
- 905 Kaufmann WE, Moser HW. 2000. Dendritic anomalies in disorders associated with mental
906 retardation. *Cereb Cortex N Y N 1991* **10**:981–991. doi:10.1093/cercor/10.10.981
- 907 Kim J, Lee S, Tsuda S, Zhang X, Asrican B, Gloss B, Feng G, Augustine GJ. 2014.
908 Optogenetic Mapping of Cerebellar Inhibitory Circuitry Reveals Spatially Biased
909 Coordination of Interneurons via Electrical Synapses. *Cell Rep* **7**:1601–1613.

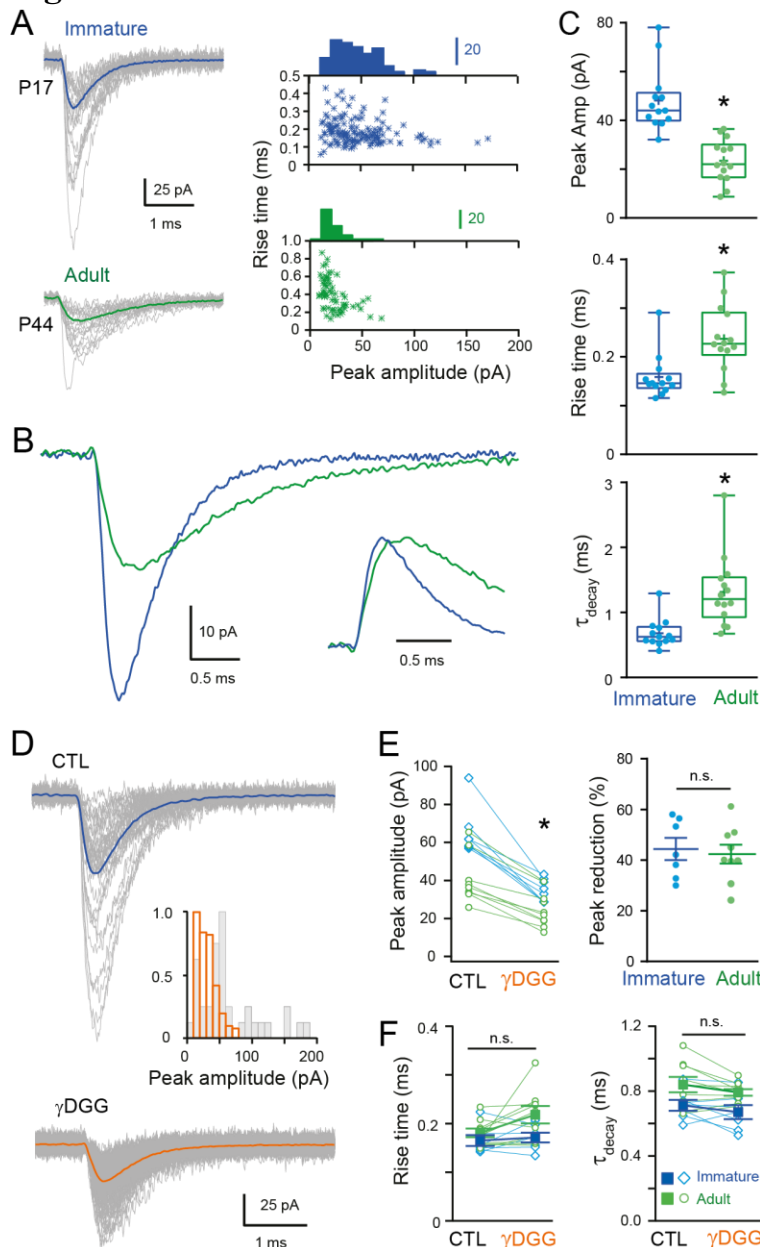
- 910 doi:10.1016/j.celrep.2014.04.047
- 911 Koike-Tani M, Saitoh N, Takahashi T. 2005. Mechanisms underlying developmental speeding
912 in AMPA-EPSC decay time at the calyx of Held. *J Neurosci* **25**:199–207.
- 913 Kwon H-B, Sabatini BL. 2011. Glutamate induces de novo growth of functional spines in
914 developing cortex. *Nature* **474**:100–104. doi:10.1038/nature09986
- 915 Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J. 2009. Synaptic integration in tuft
916 dendrites of layer 5 pyramidal neurons: a new unifying principle. *Science* **325**:756–760.
917 doi:10.1126/science.1171958
- 918 Lawrence JJ, Trussell LO. 2000. Long-term specification of AMPA receptor properties after
919 synapse formation. *J Neurosci Off J Soc Neurosci* **20**:4864–4870.
- 920 Le Magueresse C, Monyer H. 2013. GABAergic interneurons shape the functional maturation
921 of the cortex. *Neuron* **77**:388–405. doi:10.1016/j.neuron.2013.01.011
- 922 Li J, Erisir A, Cline H. 2011. In vivo time-lapse imaging and serial section electron
923 microscopy reveal developmental synaptic rearrangements. *Neuron* **69**:273–286.
924 doi:10.1016/j.neuron.2010.12.022
- 925 Liu G, Choi S, Tsien RW. 1999. Variability of neurotransmitter concentration and
926 nonsaturation of postsynaptic AMPA receptors at synapses in hippocampal cultures and
927 slices. *Neuron* **22**:395–409. doi:10.1016/s0896-6273(00)81099-5
- 928 Llano I, Gerschenfeld HM. 1993. Inhibitory synaptic currents in stellate cells of rat cerebellar
929 slices. *J Physiol Lond* **468**:177–200.
- 930 Losonczy A, Magee JC. 2006. Integrative properties of radial oblique dendrites in
931 hippocampal CA1 pyramidal neurons. *Neuron* **50**:291–307.
932 doi:10.1016/j.neuron.2006.03.016
- 933 Losonczy A, Makara JK, Magee JC. 2008. Compartmentalized dendritic plasticity and input
934 feature storage in neurons. *Nature* **452**:436–441. doi:10.1038/nature06725
- 935 Magee JC. 2000. Dendritic integration of excitatory synaptic input. *Nat Rev Neurosci* **1**:181–
936 190. doi:10.1038/35044552
- 937 Magee JC. 1999. Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons.
938 *Nat Neurosci* **2**:508–514. doi:10.1038/9158
- 939 Magee JC, Cook EP. 2000. Somatic EPSP amplitude is independent of synapse location in
940 hippocampal pyramidal neurons. *Nat Neurosci* **3**:895–903. doi:10.1038/78800
- 941 Marín O. 2016. Developmental timing and critical windows for the treatment of psychiatric
942 disorders. *Nat Med* **22**:1229–1238. doi:10.1038/nm.4225
- 943 Masugi-Tokita M, Tarusawa E, Watanabe M, Molnar E, Fujimoto K, Shigemoto R. 2007.
944 Number and density of AMPA receptors in individual synapses in the rat cerebellum as
945 revealed by SDS-digested freeze-fracture replica labeling. *J Neurosci* **27**:2135–44.
- 946 McBride TJ, Rodriguez-Contreras A, Trinh A, Bailey R, DeBello WM. 2008. Learning drives
947 differential clustering of axodendritic contacts in the barn owl auditory system. *J*
948 *Neurosci Off J Soc Neurosci* **28**:6960–6973. doi:10.1523/JNEUROSCI.1352-08.2008
- 949 McCormick DA, Prince DA. 1987. Post-natal development of electrophysiological properties
950 of rat cerebral cortical pyramidal neurones. *J Physiol* **393**:743–762.
951 doi:10.1113/jphysiol.1987.sp016851
- 952 Menon V, Musial TF, Liu A, Katz Y, Kath WL, Spruston N, Nicholson DA. 2013. Balanced
953 synaptic impact via distance-dependent synapse distribution and complementary
954 expression of AMPARs and NMDARs in hippocampal dendrites. *Neuron* **80**:1451–1463.
955 doi:10.1016/j.neuron.2013.09.027

- 956 Mittmann W, Koch U, Häusser M. 2005. Feed-forward inhibition shapes the spike output of
957 cerebellar Purkinje cells. *J Physiol* **563**:369–378. doi:10.1113/jphysiol.2004.075028
- 958 Nevian T, Larkum ME, Polsky A, Schiller J. 2007. Properties of basal dendrites of layer 5
959 pyramidal neurons: a direct patch-clamp recording study. *Nat Neurosci* **10**:206–214.
960 doi:10.1038/nn1826
- 961 Nicholson DA, Trana R, Katz Y, Kath WL, Spruston N, Geinisman Y. 2006. Distance-
962 dependent differences in synapse number and AMPA receptor expression in hippocampal
963 CA1 pyramidal neurons. *Neuron* **50**:431–442. doi:10.1016/j.neuron.2006.03.022
- 964 Pelkey KA, Barksdale E, Craig MT, Yuan X, Sukumaran M, Vargish GA, Mitchell RM,
965 Wyeth MS, Petralia RS, Chittajallu R, Karlsson R-M, Cameron HA, Murata Y,
966 Colonnese MT, Worley PF, McBain CJ. 2015. Pentraxins coordinate excitatory synapse
967 maturation and circuit integration of parvalbumin interneurons. *Neuron* **85**:1257–1272.
968 doi:10.1016/j.neuron.2015.02.020
- 969 Peng Y, Lu Z, Li G, Piechowicz M, Anderson M, Uddin Y, Wu J, Qiu S. 2016. The autism-
970 associated MET receptor tyrosine kinase engages early neuronal growth mechanism and
971 controls glutamatergic circuits development in the forebrain. *Mol Psychiatry* **21**:925–
972 935. doi:10.1038/mp.2015.182
- 973 Penzes P, Cahill ME, Jones KA, VanLeeuwen J-E, Woolfrey KM. 2011. Dendritic spine
974 pathology in neuropsychiatric disorders. *Nat Neurosci* **14**:285–293. doi:10.1038/nn.2741
- 975 Poirazi P, Mel BW. 2001. Impact of active dendrites and structural plasticity on the memory
976 capacity of neural tissue. *Neuron* **29**:779–96.
- 977 Polsky A, Mel BW, Schiller J. 2004. Computational subunits in thin dendrites of pyramidal
978 cells. *Nat Neurosci* **7**:621–627. doi:10.1038/nn1253
- 979 Pouille F, Scanziani M. 2001. Enforcement of Temporal Fidelity in Pyramidal Cells by
980 Somatic Feed-Forward Inhibition. *Science* **293**:1159–1163. doi:10.1126/science.1060342
- 981 Rall W. 1967. Distinguishing theoretical synaptic potentials computed for different soma-
982 dendritic distributions of synaptic input. *J Neurophysiol* **30**:1138–1168.
983 doi:10.1152/jn.1967.30.5.1138
- 984 Rodriguez A, Ehlenberger DB, Dickstein DL, Hof PR, Wearne SL. 2008. Automated three-
985 dimensional detection and shape classification of dendritic spines from fluorescence
986 microscopy images. *PloS One* **3**:e1997. doi:10.1371/journal.pone.0001997
- 987 Rothman JS, Silver RA. 2018. NeuroMatic: An Integrated Open-Source Software Toolkit for
988 Acquisition, Analysis and Simulation of Electrophysiological Data. *Front*
989 *Neuroinformatics* **12**:14. doi:10.3389/fninf.2018.00014
- 990 Sanes DH. 1993. The development of synaptic function and integration in the central auditory
991 system. *J Neurosci Off J Soc Neurosci* **13**:2627–2637.
- 992 Schiller J, Major G, Koester HJ, Schiller Y. 2000. NMDA spikes in basal dendrites of cortical
993 pyramidal neurons. *Nature* **404**:285–289. doi:10.1038/35005094
- 994 Scholl B, Wilson DE, Fitzpatrick D. 2017. Local Order within Global Disorder: Synaptic
995 Architecture of Visual Space. *Neuron* **96**:1127–1138.e4.
996 doi:10.1016/j.neuron.2017.10.017
- 997 Sigler A, Oh WC, Imig C, Altas B, Kawabe H, Cooper BH, Kwon H-B, Rhee J-S, Brose N.
998 2017. Formation and Maintenance of Functional Spines in the Absence of Presynaptic
999 Glutamate Release. *Neuron* **94**:304–311.e4. doi:10.1016/j.neuron.2017.03.029
- 1000 Silver RA. 2010. Neuronal arithmetic. *Nat Rev Neurosci* **11**:474–489. doi:10.1038/nrn2864
- 1001 Taschenberger H, von Gersdorff H. 2000. Fine-tuning an auditory synapse for speed and

- 1002 fidelity: developmental changes in presynaptic waveform, EPSC kinetics, and synaptic
1003 plasticity. *J Neurosci Off J Soc Neurosci* **20**:9162–9173.
- 1004 Tia S, Wang JF, Kotchabhakdi N, Vicini S. 1996. Developmental changes of inhibitory
1005 synaptic currents in cerebellar granule neurons: role of GABA(A) receptor alpha 6
1006 subunit. *J Neurosci Off J Soc Neurosci* **16**:3630–3640.
- 1007 Tran-Van-Minh A, Caze RD, Abrahamsson T, Cathala L, Gutkin BS, DiGregorio DA. 2015.
1008 Contribution of sublinear and supralinear dendritic integration to neuronal computations.
1009 *Front Cell Neurosci* **9**:67. doi:10.3389/fncel.2015.00067
- 1010 Tzilivaki A, Kastellakis G, Poirazi P. 2019. Challenging the point neuron dogma: FS basket
1011 cells as 2-stage nonlinear integrators. *Nat Commun* **10**:1–14. doi:10.1038/s41467-019-
1012 11537-7
- 1013 Vervaeke K, Lorincz A, Nusser Z, Silver RA. 2012. Gap junctions compensate for sublinear
1014 dendritic integration in an inhibitory network. *Science* **335**:1624–1628.
1015 doi:10.1126/science.1215101
- 1016 Vlasits AL, Morrie RD, Tran-Van-Minh A, Bleckert A, Gainer CF, DiGregorio DA, Feller
1017 MB. 2016. A Role for Synaptic Input Distribution in a Dendritic Computation of Motion
1018 Direction in the Retina. *Neuron* **89**:1317–1330. doi:10.1016/j.neuron.2016.02.020
- 1019 Wei W, Hamby AM, Zhou K, Feller MB. 2011. Development of asymmetric inhibition
1020 underlying direction selectivity in the retina. *Nature* **469**:402–406.
1021 doi:10.1038/nature09600
- 1022 Williams SR, Stuart GJ. 2002. Dependence of EPSP efficacy on synapse location in
1023 neocortical pyramidal neurons. *Science* **295**:1907–1910. doi:10.1126/science.1067903
- 1024 Wilms CD, Häusser M. 2015. Reading out a spatiotemporal population code by imaging
1025 neighbouring parallel fibre axons in vivo. *Nat Commun* **6**:6464.
1026 doi:10.1038/ncomms7464
- 1027 Xu N, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, Magee JC. 2012.
1028 Nonlinear dendritic integration of sensory and motor input during an active sensing task.
1029 *Nature* **492**:247–251. doi:10.1038/nature11601
- 1030 Yamashita T, Ishikawa T, Takahashi T. 2003. Developmental increase in vesicular glutamate
1031 content does not cause saturation of AMPA receptors at the calyx of held synapse. *J*
1032 *Neurosci* **23**:3633–8.
- 1033 Zhang Z. 2004. Maturation of layer V pyramidal neurons in the rat prefrontal cortex: intrinsic
1034 properties and synaptic function. *J Neurophysiol* **91**:1171–1182.
1035 doi:10.1152/jn.00855.2003
- 1036
- 1037

1038

Figure 1



1039

1040

1041

1042

1043

1044

1045

1046

1047

1048

1049

1050

1051

1052

1053

1054

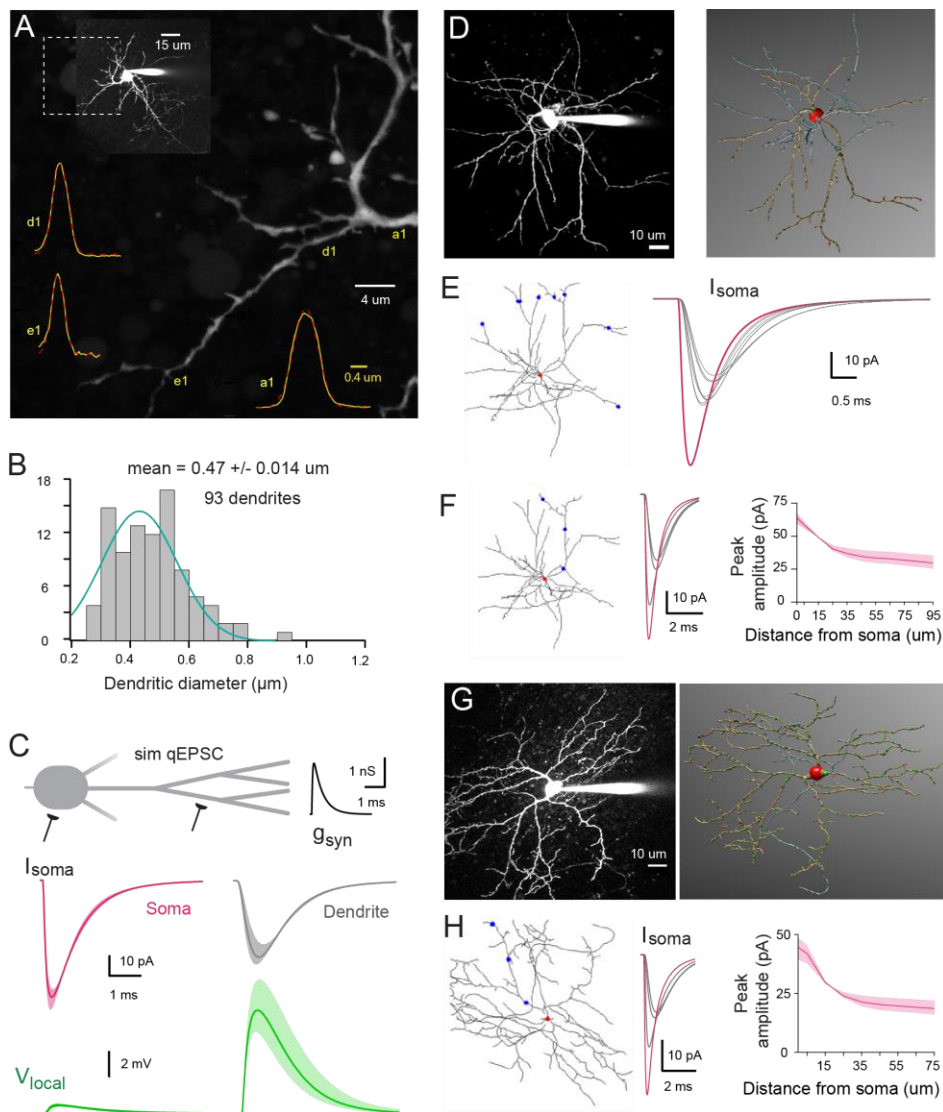
1055

1056

Figure 1. Developmental maturation of the AMPAR-mediated mEPSC in SC. (A) Left panels show superimposed single mEPSCs (grey) and the corresponding average (bold) recorded at -70 mV obtained from a representative immature SCs (P17, blue trace) and adult SCs (P44, green trace). Right panels show corresponding plots of peak amplitude as a function of 10-90% rise time, with the superimposed amplitude distributions. (B) Superimposed mEPSC average aligned on event onset. Inset : traces normalized to their peak (C) Box and whisker plots showing the median (line) peak amplitude, 10-90% rise time and decay (τ_{decay}) at both ages (PN 17 to 19; 17.4 ± 0.27 , days, $n = 13$ and PN 37 to 57; 45 ± 1.75 days, $n = 14$, $n = 14$ respectively), the 25th and 75th percentile (box), range (whiskers) and mean (+). Superimposed filled circles represent individual cells (asterisks denote $P < 0.05$). (D) Representative examples of single mEPSC events (grey) and the corresponding average (bold) in control (CTL) and in the presence of γ DGG (1 mM). Inset shows the corresponding mEPSC peak amplitude distributions. (E) The effect of γ DGG on mEPSCs at the two ages ($n = 7$ and $n = 9$ respectively): left panel its effect on individual mean peak amplitude for each SC (blue for immature and green for adult SC); and right panel, summary plot showing the % reduction of mEPSC peak amplitude. (F) Plot summarizing the effect of γ DGG on mEPSC rise time (left panel) and decay (τ_{decay} , right panel) at the two ages for individual cells (open symbols) and on population averages (\pm SEM)

1057 **Figure 2**

1058



1059

1060

1061 **Figure 2. Numerical simulations of SC dendrites indicate significant cable filtering and large**

1062 **local depolarizations in immature SC. (A)** Maximal intensity projection of one-photon confocal

1063 images of an immature SC labeled with Alexa 488. Examples of intensity profiles (Yellow line) of 3

1064 dendritic locations superimposed on the image. Dendrite diameter is approximated by the full width

1065 half maximum (FWHM) of the Gaussian fit of the line profile (broken red line). **(B)** Histogram

1066 showing the distribution of dendrite diameters from 93 dendrites, with a Gaussian fit indicating a

1067 mode centered at $0.43 \pm 0.008 \mu\text{m}$. **(C)** Numerical simulations of somatic qEPSCs in a passive neuron

1068 under voltage-clamp (with $C_m = 0.9 \text{ pF/cm}^2$, $R_m = 20,000 \Omega\text{cm}^2$, and $R_i = 150 \pm 50 \Omega\text{cm}$). The

1069 idealized SC dendritic morphology has an uniform diameter of $0.47 \mu\text{m}$ and 3 dendritic branch points.

1070 Top traces, simulated qEPSCs (sim qEPSC) in response to a quantal synaptic conductance (g_{syn})

1071 injected at the soma (magenta) and at a distance of $60 \mu\text{m}$ on a dendrite (grey traces). g_{syn} was set to

1072 reproduce the experimental quantal EPSCs following somatic synapses activation. Bottom traces

1073 (green), the corresponding local voltage transients at the site of synaptic conductance injection.

1074 Boundaries of shaded region indicate simulations with a R_i of 100 to $200 \Omega\text{cm}$. **(D)** 2PLSM image of

1075 a P16 SC (maximal intensity projection) patch- loaded with $30 \mu\text{M}$ Alexa 594 and the corresponding

1076 3D reconstruction in *NeuronStudio* (red: soma, brown: dendrite, bleu: axon). **(E)** Superimposed

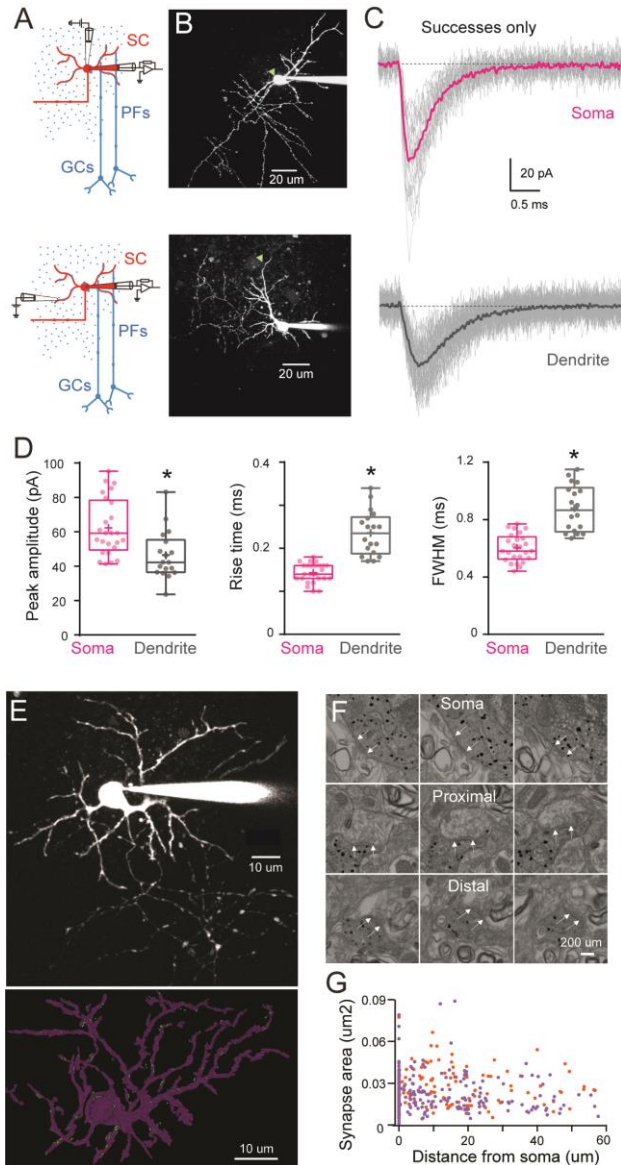
1077 numerical simulation of quantal EPSCs in the reconstructed P16 SC (with $C_m = 0.9 \text{ pF/cm}^2$, $R_m =$

1078 $20,000 \Omega\text{cm}^2$, and $R_i = 150 \Omega\text{cm}$) in response to an quantal conductance (g_{syn}) at the soma (red dot,

1079 magenta trace) or at a distance of 60 μm on 6 different dendritic branches (blue dots, grey traces). g_{syn}
1080 is set to reproduce immature qEPSCs evoked by somatic synapses. **(F)** Sim qEPSC induced when
1081 synapses are activated at the soma (red dot, magenta trace) or on a single dendrite (blue dot, grey
1082 traces) Summary plot showing the sim qEPSC amplitude as a function of synaptic location along the
1083 somato-dendritic compartment. Boundaries of shaded region indicate simulations with a Ri of 100 to
1084 200 Ωcm . **(G)** Same as in (D) but for a P42 SC. **(H)** same as in (F) but with the reconstructed P42 SC
1085 and g_{syn} to reproduce experimental adult somatic qEPSC.
1086

1087

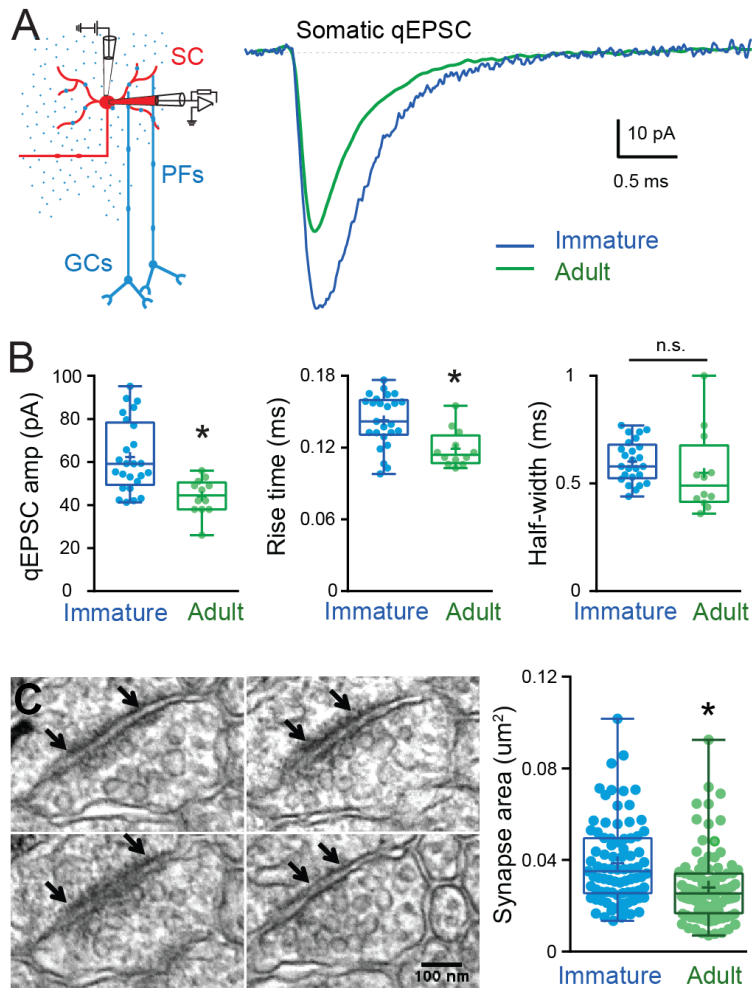
Figure 3



1088 **Figure 3. Difference in quantal EPSC properties and PSD area between the soma and dendrite**
 1089 **in immature SC.** (A) Diagram of a parasagittal cerebellar slice showing parallel fibers (PFs)
 1090 projecting perpendicular (blue dots) to the dendritic plane of SCs (in red), allowing precise
 1091 positioning of the stimulus electrode with respect to the soma (top panel) or an isolated SC dendrite
 1092 (lower panel). (B) 2PLSM images (maximal intensity projection) of two immature SC loaded with the
 1093 patch-pipette with 30 μ M Alexa 594 with the location of stimulating pipette indicated with a green
 1094 triangle. (C) Superimposed single qEPSCs (grey) from *successful trials*, and the corresponding
 1095 averaged traces (bold) recorded in response to extracellular stimulation at the soma (top) and on a
 1096 dendrite (bottom) under low release probability conditions (external $[Ca^{2+}]/[Mg^{2+}]$ was 0.5/5 mM;
 1097 failure rate >90%). (D) Box and whisker plots showing the median (line) peak amplitude, 10-90% rise
 1098 time and full-width half maximum (half-width) following somatic (magenta) or dendritic (grey)
 1099 synapses activation ($n = 25$ and $n = 18$ respectively), the 25th and 75th percentile (box), range
 1100 (whiskers) and mean (+). Superimposed filled circle represent individual cells (asterisks denote
 1101 $P < 0.05$). (E) 2PLSM image of an Alexa 594 loaded P14 SC (maximal intensity projection) before
 1102 fixation and (below) its 3D rendering after an EM reconstruction. Light dots indicate PSD locations.
 1103 (F) Electron micrographs of an immunogold labeled SC soma with proximal and distal dendritic
 1104 segments. The outer bound of excitatory synapses are indicated by arrows. Scale bar, 200nm (G) Plot
 1105 of synapse area versus distance from soma. Orange and purple circles indicate data obtained from two
 1106 immature SC (P14, $n = 172$ synapses and P17 $n = 220$ synapses - total $n = 393$).

1107 **Figure 4**

1108



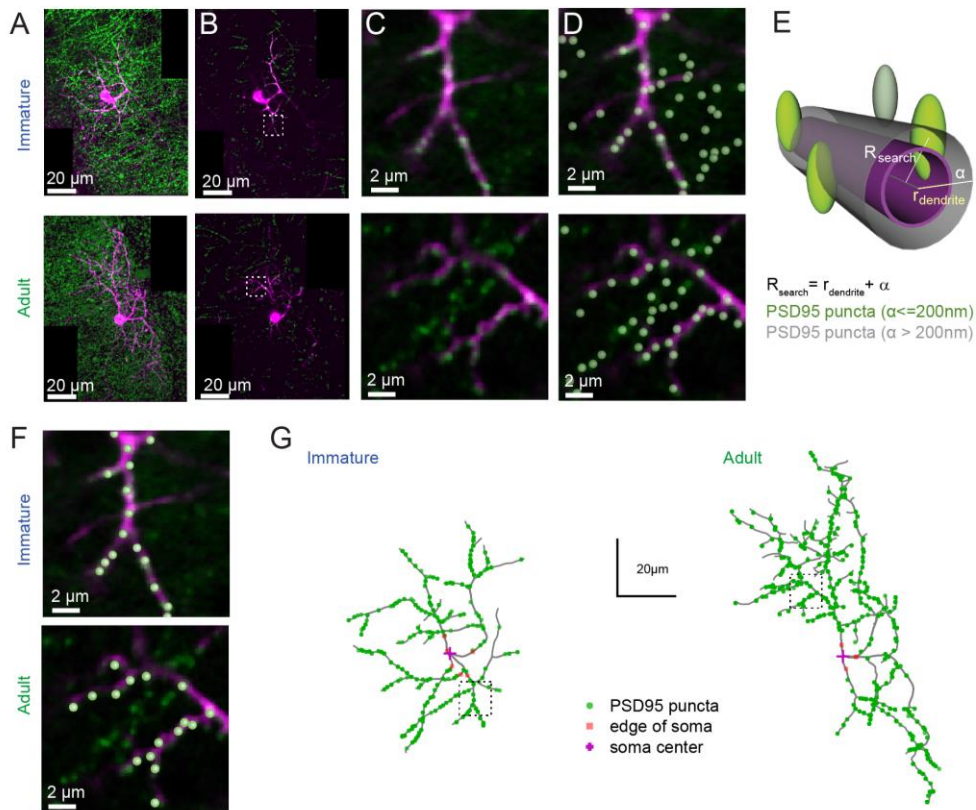
1109

1110 **Figure 4. Developmental changes in somatic qEPSC properties and somatic PSD size.** (A) Left
1111 panel shows a diagram of a parasagittal cerebellar slice showing parallel fibers (PFs) projecting
1112 perpendicular (blue dots) to the dendritic plane of a SC (in red), showing the stimulus electrode (top)
1113 position above the soma. Right panel shows superimposition of representative qEPSC averages
1114 aligned on event onset, from immature SC (blue trace) and adult SC (green trace, from Abrahamson *et*
1115 *al.*, 2012). (B) Summary box and whisker plot showing the qEPSC amplitude, 10-90% rise time and
1116 half-width for immature (blue, n = 25) and adult (green, n = 12) SC (asterisks denote $P < 0.05$). (C)
1117 Serial electron micrographs of an asymmetrical synapse made by axon terminals on an immature SC
1118 soma. Right panel : Summary box and whisker plot showing the synapse area obtained from immature
1119 (n = 83 synapses from 3 cells) and adult SC (n = 97 synapses from 2 cells) somata. Superimposed
1120 filled circles represent individual synapses (asterisks denote $P < 0.05$). The outer boundaries of an
1121 excitatory synapse are indicated by arrows. Scale bar, 100 nm.

1122

1123

1124 **Figure 5**



1125

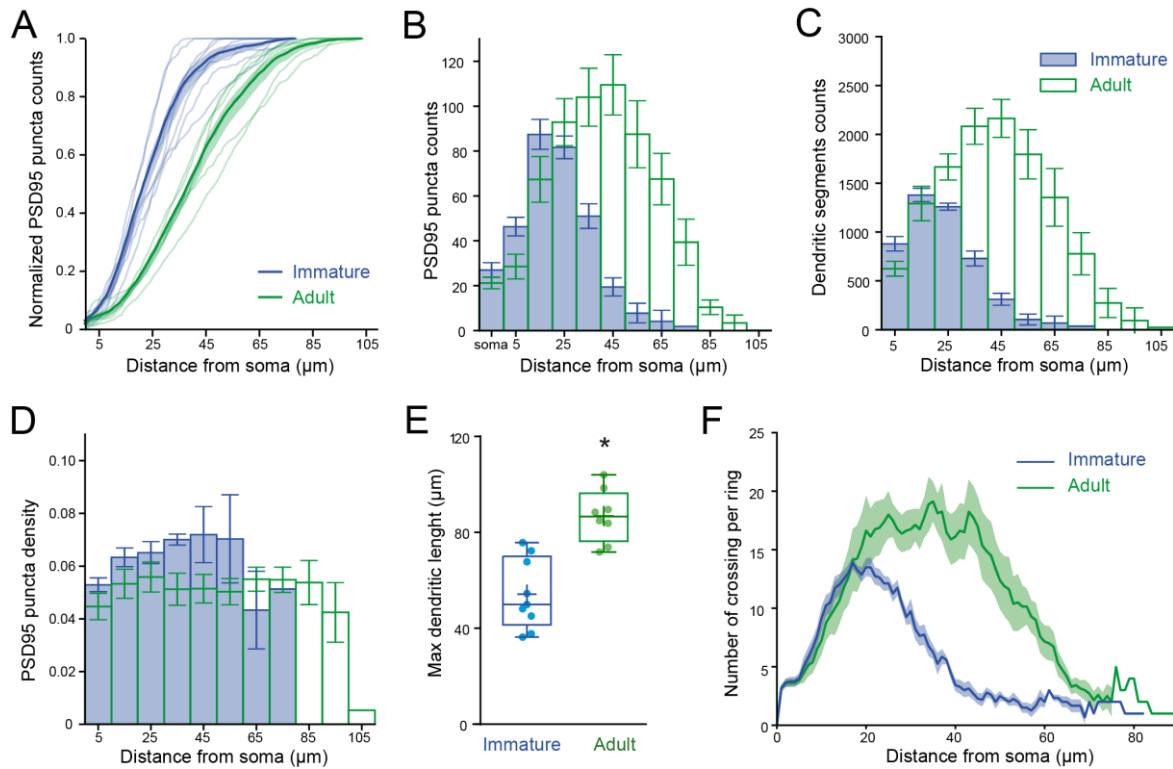
1126 **Figure 5: PSD95 puncta distribution on immature and adult SC.** (A) Maximum intensity
1127 projection of merged images showing a P14 (top) and P42 (bottom) SC labelled with Alexa594
1128 (magenta) and venus-tagged PSD95 puncta (green). (B) Example of single optical sections from (A).
1129 (C) Inset indicated in (B) showing details of a dendritic branch with venus-tagged PSD95 puncta. (D)
1130 Detected PSD95 puncta on the same focal plane overlaid on the fluorescent image. (E) Diagram
1131 describing the criteria for assignment of PSD95 puncta to a dendrite branch. Puncta were considered
1132 as associated with the dendrite if their centers were located within a search radius (R_{search}) defined as
1133 the local dendritic radius $r_{\text{dendrite}} + \alpha$, where $\alpha = 0.2\mu\text{m}$. (F) Examples of detected PSD95 puncta
1134 identified as connected to dendritic structure in an immature (top) and adult (bottom) SC. (G)
1135 Skeleton representation of the dendritic tree of an immature (left) and adult (right) SC with detected
1136 PSD95 puncta in green. The edge and the center of the soma are indicated by orange squares and
1137 magenta crosses, respectively.

1138

1139

1140

1141 **Figure 6**



1142

1143

1144 **Figure 6: PSD95 puncta distribution and morphological analysis on immature and adult SC. (A)**

1145 Cumulative plot showing venus-tagged PSD95 puncta distribution for individual and average (bold

1146 traces with boundaries of shaded region indicate SEM) obtained from 9 immature (blue) and 8 adult

1147 (green) SC ($P < 0.05$, KS test). **(B)** Superimposed histograms of the mean PSD95 puncta count on the

1148 soma and dendrites of immature (blue) and adult (green) SC with a 10 μm increment. **(C)**

1149 Superimposed histograms of the average dendritic segment count (segment length of 100 nm) for

1150 immature (blue) and adult (green) SCs. **(D)** Dendritic mean PSD95 puncta density as a function of

1151 distance estimated from B and C (for all $P > 0.05$ except at 35 μm; MW test). **(E)** Summary box and

1152 whisker plot showing the maximal dendritic length per neuron for immature and adult SC.

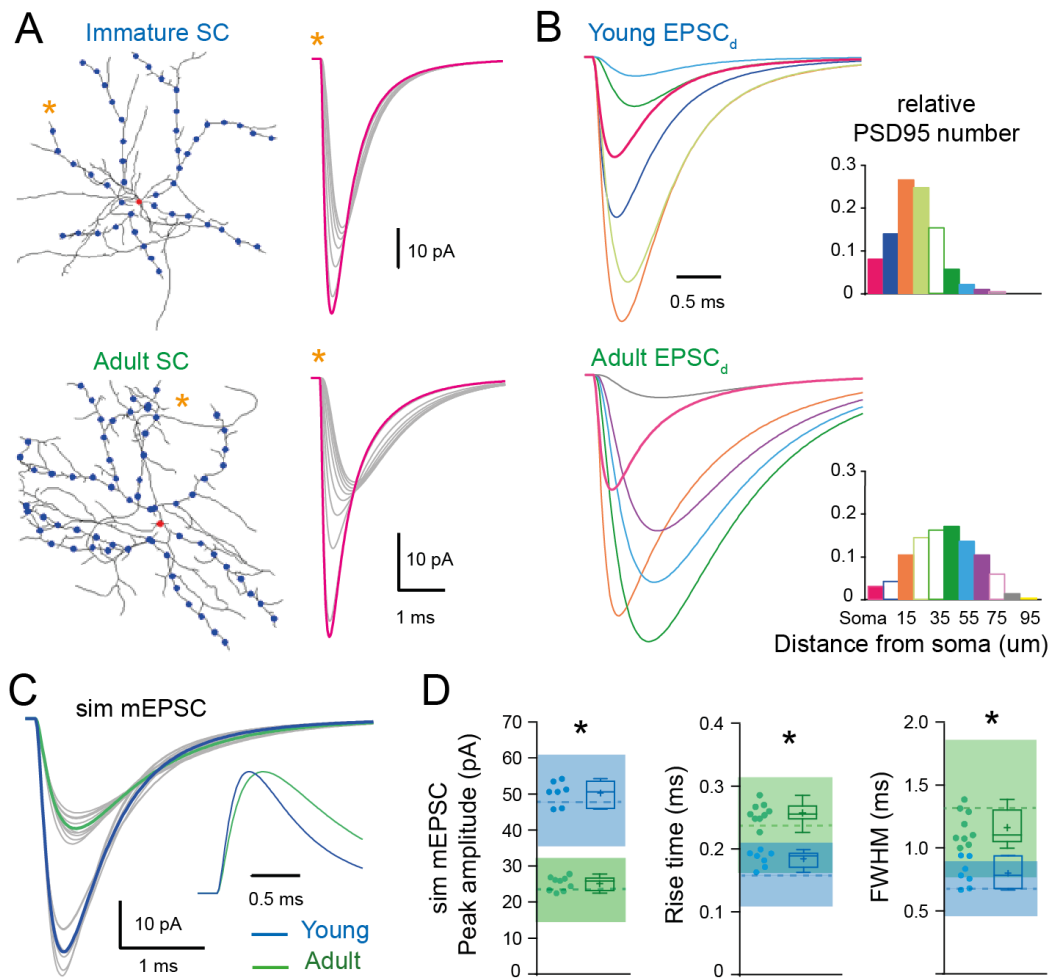
1153 Superimposed filled circle represent individual cells (asterisks denote $P < 0.05$). **(F)** Sholl analysis

1154 showing increased arbor complexity with development.

1155

1156

1157 **Figure 7**

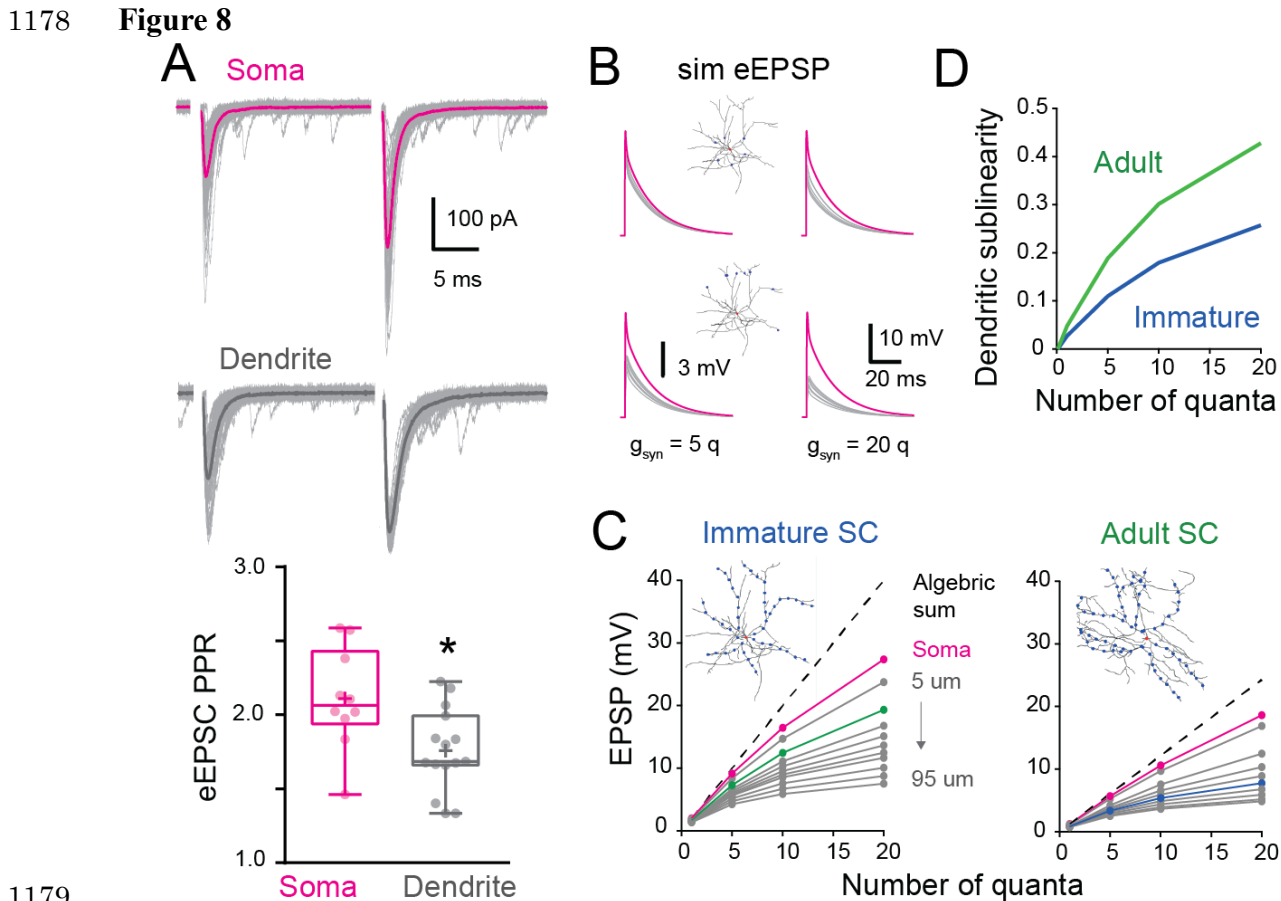


1158

1159 **Figure 7. The developmental change in synaptic distribution recapitulates the developmental**
 1160 **change in mEPSC properties (A)** Numerical simulations of somatic and dendritic qEPSCs for
 1161 synapses placed on the soma (red dot) and at 10 μm intervals (blue dots) along 7 of the longest
 1162 dendrites of a reconstructed immature (P16; top) and 9 of the longest dendrites of a reconstructed
 1163 adult (P42; bottom) SC (with $C_m = 0.9 \text{ pF/cm}^2$, $R_m = 20,000 \Omega\text{cm}^2$, $R_i = 150 \pm 50 \Omega\text{cm}$). Right panel
 1164 shows example of qEPSC for somatic (magenta) and dendritic (grey traces) synapses along a single
 1165 dendrite labeled with an asterisk. The synaptic quantal conductances g_{syn} were set to reproduce the
 1166 experimental qEPSCs when stimulating somatic synapses at both ages. **(B)** The relative frequency of
 1167 the excitatory synapse distribution (right panel) was used to scale each qEPSC to produce a
 1168 normalized qEPSC_d describing its relative contribution of the mean mEPSC waveform. **(C)**
 1169 Superimposed mEPSC waveform obtained from added the qEPSC_d for each dendrites (grey) and the
 1170 corresponding average mEPSC (bold) obtained from the immature SC (P17, blue trace) and adult SC
 1171 (P44, green trace). Inset: traces normalized to their peak. **(D)** Summary box and whisker plots
 1172 showing the median (line) peak amplitude, 10-90% rise time and decay, the 25th and 75th percentile
 1173 (box), range (whiskers) and mean (+) of the sim mEPSC for the immature (blue) and adult (green)
 1174 SCs. Individual dendritic mEPSCs are illustrated with filled circles (asterisks denote $P < 0.05$). Dotted
 1175 line shows the experimental mEPSC average values ± 1 SD (shaded region).

1176

1177



1179

1180 **Figure 8 : Location dependence of short-term plasticity and sublinear behavior in immature SC**
 1181 **(A)** Superimposed recorded single evoked EPSCs (grey) and the corresponding average (bold) in
 1182 response to a pair of extracellular stimuli (50hz) when the stimulation electrode is placed above the
 1183 soma (magenta) and distally on an isolated dendrite (grey). Bottom: summary box and whisker plot of
 1184 paired-pulse ratio of EPSC amplitudes ($PPR = EPSC_2/EPSC_1$) for somatic synapses ($n = 10$) and
 1185 dendritic synapses ($n = 15$). The PPR ratio was assessed from recordings where the first EPSC had an
 1186 amplitude inferior to 400 pA and had a failure rate below 30% (for somatic synapses, $Amp = 239.4 \pm$
 1187 32 pA with a half-width = 0.88 ± 0.07 ms, $n = 10$). Superimposed filled circles represent individual
 1188 cells (asterisks denote $P < 0.05$). **(B)** Simulated evoked EPSP (sim eEPSP) under current-clamp
 1189 conditions for synapses at the soma (magenta) and at 20 or 60 μm on dendrites (grey) of a
 1190 reconstructed immature SC. g_{syn} peak amplitude is set at 5 or 20 quanta *ie* is a multiple of the g_{syn} that
 1191 produce a qEPSC for somatic synapses. **(C)** Subthreshold input-output relationship of sim eEPSPs
 1192 obtained by plotting the average peak sim eEPSP amplitude for an increase number of quanta as the
 1193 function the algebraic sum of the sim eEPSP of a reconstructed immature (left) and adult (right) SC.
 1194 The dotted black line has a slope of 1. The circles indicate sim eEPSP resulting from a g_{syn} peak
 1195 amplitude of 1, 5, 10 and 20 quanta. **(D)** Summary plot showing dendritic sublinearity ($1 -$ (dendritic
 1196 EPSP amp/soma EPSP amp) with EPSP converted to number of quanta) as a function of number of
 1197 quanta for reconstructed immature (blue) and adult (green) SCs.

1198