

1 **Gradients in the cerebellar cortex enable Fourier-like transformation and**  
2 **improve storing capacity**

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19

20 **Abstract**

21 Cerebellar granule cells (GCs) making up majority of all the neurons in the  
22 vertebrate brain, but heterogeneities among GCs and potential functional  
23 consequences are poorly understood. Here, we identified unexpected gradients  
24 in the biophysical properties of GCs. GCs closer to the white matter (inner-zone  
25 GCs) had higher firing thresholds and could sustain firing with larger current  
26 inputs. Dynamic clamp experiments showed that inner- and outer-zone GCs  
27 preferentially respond to high- and low-frequency mossy fiber inputs,  
28 respectively, enabling to disperse the mossy fiber input into its frequency  
29 components as performed by a Fourier transformation. Furthermore, inner-zone  
30 GCs have faster axonal conduction velocity and elicit faster synaptic potentials in  
31 Purkinje cells. Neuronal network modeling revealed that these gradients improve  
32 spike-timing precision of Purkinje cells and decrease the number of GCs required  
33 to learn spike-sequences. Thus, our study uncovers biophysical gradients in the  
34 cerebellar cortex enabling a Fourier-like transformation of mossy fiber inputs.

## 35 **Introduction**

36 Digital audio compression (e.g., 'MP3'; Jayant et al., 1993) and image  
37 compression (e.g., 'JPEG'; Wallace, 1992) rely on Fourier transformations, which  
38 decompose a signal (e.g., sound amplitude as a function of time or image  
39 intensity as a function of space) into its frequency components (power as a  
40 function of frequency). By storing these frequency components with different  
41 precision depending on psychophysical demands of hearing and seeing, the  
42 overall storage capacity can be increased dramatically. In principle, neuronal  
43 networks consisting of neurons with varied electrophysiological properties could  
44 be suitable for Fourier-like transformations of information. This could benefit  
45 processing in neuronal circuits by increasing the signal-to-noise ratio of input  
46 signals or by selecting only relevant spectral components of a signal.  
47 Interestingly, there are indications that for example pyramidal neurons in visual  
48 cortex and in the hippocampus are tuned to different inputs or different input  
49 strengths (Cembrowski and Spruston, 2019; Fletcher and Williams, 2019; Soltesz  
50 and Losonczy, 2018). However, whether these neuronal networks perform a  
51 Fourier-like transform on their inputs remains unknown.

52 Controlling the timing and precision of movements is considered to be one of the  
53 main functions of the cerebellum. In the cerebellum, the firing frequency of  
54 Purkinje cells (PCs) (Heiney et al., 2014; Herzfeld et al., 2015; Hewitt et al.,  
55 2011; Medina and Lisberger, 2007; Payne et al., 2019; Sarnaik and Raman,  
56 2018; Witter et al., 2013) or the timing of spikes (Brown and Raman, 2018;  
57 Sarnaik and Raman, 2018) have been shown to be closely related to movement.  
58 Indeed, cerebellar pathology impairs precision in motor learning tasks (Gibo et  
59 al., 2013; Martin et al., 1996) and timing of rhythmic learning tasks (Keele and  
60 Ivry, 1990). These functions are executed by a remarkably simple neuronal  
61 network architecture. Inputs from mossy fibers (MFs) are processed by GCs and  
62 transmitted via their parallel fiber (PF) axons to PCs, which provide the sole  
63 output from the cerebellar cortex. GCs represent the first stage in cerebellar  
64 processing and have been proposed to provide pattern separation and

65 conversion into a sparser representation of the MF input (recently reviewed by  
66 (Cayco-Gajic and Silver, 2019). These MF inputs show a wide variety of signaling  
67 frequencies, ranging from slow modulating activity to kilohertz bursts of activity  
68 (Arenz et al., 2008; Rancz et al., 2007; Ritzau-Jost et al., 2014; van Kan et al.,  
69 1993). Interestingly, in cellular models of the cerebellum, each MF is considered  
70 to be either active or inactive with little consideration for this wide range of  
71 frequencies (Albus, 1971; Marr, 1969). Furthermore, in these models, GCs are  
72 generally considered as a uniform population of neurons.

73 Here we show that the biophysical properties of GCs differ according to their  
74 vertical position in the GC layer. GCs located close to the white matter (inner-  
75 zone) selectively transmit high-frequency MF inputs, have shorter action  
76 potentials and a higher voltage threshold to fire an action potential compared with  
77 GCs close to the PC layer (outer-zone). This gradient of GC properties enables a  
78 Fourier-like transformation of the MF input, where inner-zone GCs convey the  
79 high-frequency and outer-zone GCs the low-frequency components of the MF  
80 input. These different Fourier-like components are sent to PCs by specialized  
81 downstream signaling pathways, differing in PF axon diameters, action potential  
82 velocity and PC excitatory postsynaptic potential (EPSP) kinetics. Computational  
83 simulations show that the biophysical gradients in the GC and molecular layer  
84 significantly reduce the number of GCs required to learn a sequence of firing  
85 frequencies and accelerate the time required to switch between firing  
86 frequencies.

87

## 88 **Results**

### 89 **A gradient in the biophysical properties of inner- to outer-zone GCs**

90 To investigate whether GCs are tuned for different frequencies we first  
91 investigated the intrinsic membrane properties of GCs from different depths  
92 within the GC layer in lobule V of the cerebellum of P21-30 mice. We divided the  
93 GC layer into three zones and performed whole-cell current-clamp recordings

94 from inner- (closest to the white matter), middle- and outer-zone (closest to PCs)  
95 GCs (**Figure 1A,B**). Upon current injection, inner-zone GCs were less excitable  
96 compared with outer-zone GCs (**Figure 1C**). On average, the relationship  
97 between the mean number of action potentials and the injected current were  
98 surprisingly different for inner- and outer-zone GCs (**Figure 1D**): inner-zone GCs  
99 needed higher current injections to fire an action potential (inner:  $56.8 \pm 2.6$  pA  
100 vs. middle:  $51.2 \pm 2.0$  pA vs. outer:  $39.4 \pm 2.0$  pA;  $n = 38, 31,$  and  $38,$   
101 respectively;  $P_{ANOVA} < 0.0001$ ; **Figure 1E**) and to achieve the maximum firing rate  
102 compared with middle- and outer-zone GCs (inner:  $224.6 \pm 9.8$  pA vs. middle:  
103  $190.8 \pm 9.6$  pA vs. outer:  $174.3 \pm 9.0$  pA, respectively;  $P_{ANOVA} = 0.0007$ ; **Figure**  
104 **1F**). Consistently, inner-zone GCs had a more depolarized threshold for action  
105 potential generation compared with middle- and outer-zone GCs ( $-38.0 \pm 0.7$  mV  
106 vs.  $-38.2 \pm 0.8$  mV vs.  $-41.4 \pm 0.6$  mV;  $P_{ANOVA} = 0.001$ ; **Figure 1G**) and a lower  
107 input resistance ( $486 \pm 27$  M $\Omega$  vs.  $494 \pm 27$  M $\Omega$  vs.  $791 \pm 63$  M $\Omega$ ;  $P_{ANOVA} =$   
108  $<0.0001$ ; **Figure 1H**). Furthermore, the capacitance of inner-zone GCs was  
109 significantly larger compared to the outer-zone GCs (inner:  $5.8 \pm 0.2$  pF vs.  
110 middle:  $5.8 \pm 0.2$  pF vs outer:  $4.6 \pm 0.1$  pF;  $P_{ANOVA} = <0.0001$  **Figure 1I**). In  
111 agreement with these findings we observed depolarization block in inner-zone  
112 GCs at higher current inputs than for outer-zone GCs (**Figure 1C,D**).  
113 Furthermore, a larger delay of the first spike was observed in inner- compared  
114 with outer-zone GCs ( $P_{T-Test} = 0.01$ ; **Figure 1J**;  $P_{ANOVA} = 0.0001$ ; **Figure 1K**). The  
115 delay with 60 pA current injection was  $48 \pm 6$  ms for inner-,  $38 \pm 4$  ms for middle-,  
116 and  $23 \pm 2$  ms for outer-zone GCs ( $n = 32, 25,$  and  $37,$  respectively; note that 6  
117 out of 38 inner-zone GC did not fire an action potential at 60 pA). Finally, the  
118 action potential half-width of GCs differed significantly between the three zones  
119 (inner:  $122 \pm 2$   $\mu$ s vs. middle:  $137 \pm 4$   $\mu$ s vs. outer:  $143 \pm 4$   $\mu$ s;  $P_{ANOVA} = 0.0001$ ;  
120 **Figure 1L**).

121 To test whether these gradients are specific to lobule V, we investigated GCs in  
122 lobule IX. Here, we observed very similar gradients to lobule V (**Figure 1—figure**  
123 **supplement 1**). In short, outer-zone GCs were more excitable and had broader  
124 spikes compared with inner-zone GCs. Interestingly, the absolute values

125 between lobule V and IX differed (**Figure 1—figure supplement 1**), consistent  
126 with previously described differences in, e.g., the firing frequency *in vivo* between  
127 these two lobules (Witter and De Zeeuw, 2015a; Zhou et al., 2014) and in the  
128 differential density of Kv4 and Cav3 channel expression in GCs across different  
129 lobules (Heath et al., 2014; Rizwan et al., 2016; Serôdio and Rudy, 1998). Taking  
130 the large functional difference between spino- and vestibulo-cerebellum into  
131 account (Witter and De Zeeuw, 2015b), these data suggest that different  
132 biophysical properties of GCs is likely a conserved mechanism throughout the  
133 entire cerebellar cortex, potentially tuning GCs to different frequencies.

134 Development can have large effects on the physiology of neurons, and GCs in  
135 particular undergo profound changes during development (Dhar et al., 2018;  
136 Lackey et al., 2018). To exclude confounding effects of developmental stage, we  
137 tested whether these gradients were also present at a later developmental stage.  
138 Recordings obtained from GCs in lobule V in animals between 80 and 100 days  
139 of age showed the very similar gradients as in young animals (**Figure 1—figure  
140 supplement 2**). Together, these data show a prominent gradient in the  
141 electrophysiological properties of GCs over the depth of the granule cell layer,  
142 and that this gradient can consistently be found in different lobules and ages.

#### 143 **Voltage-gated potassium currents are larger at inner-zone GCs**

144 To investigate possible causes for the gradient in the biophysical properties, we  
145 investigated voltage-gated potassium (Kv) currents by performing voltage-clamp  
146 recordings in outside-out patches from somata of inner- and outer-zone GCs in  
147 lobule V (**Figure 2A**). The maximum Kv current was significantly higher in inner-  
148 zone GCs ( $282 \pm 29$  pA,  $n = 48$ ) compared with outer-zone GCs ( $221 \pm 28$  pA,  $n$   
149  $= 54$ ,  $P_{\text{Mann-Whitney}} = 0.02$ ; **Figure 2B**). Neither the steady-state activation curve  
150 (**Figure 2C**) nor the degree of inactivation (**Figure 2D**) was different between the  
151 two GC populations. Furthermore, steady-state inactivation, which was  
152 investigated with different holding potentials, was similar between inner- and  
153 outer-zone GCs (**Figure 2—figure supplement 1**). These data suggest that  
154 inner- and outer-zone GCs have a similar composition of Kv channels, but inner-

155 zone GCs have a higher Kv channel density. The here observed larger Kv  
156 currents in inner-zone GCs are consistent with the short action potential duration  
157 of inner-zone GCs (cf. Figure 1). Thus, our data provide a biophysical  
158 explanation for the observed gradient in GC properties.

### 159 **MF inputs are differentially processed by inner and outer GCs**

160 The gradient within the GC layer creates an optimal range of input strengths for  
161 each GC. To test how this gradient impacts the processing of synaptic MF inputs,  
162 we performed dynamic clamp experiments to test whether different MF input  
163 frequencies differentially affect spiking in inner- and outer-zone GCs (**Figure 3A**).  
164 We first recorded excitatory postsynaptic currents (EPSC) from GCs located at  
165 inner- or outer-zone of lobule V after single MF stimulation. We found no  
166 significant differences in the amplitude nor in the kinetics of EPSCs in inner- and  
167 outer-zone GCs (**Figure 3—figure supplement 1**).

168 Individual MFs span the entire depth of the GC layer, contacting both inner- and  
169 outer-zone GCs (Krieger et al., 1985; Palay and Chan-Palay, 1974).  
170 Furthermore, GCs are electronically extremely compact neurons and can be  
171 considered as a single compartment (D'Angelo et al., 1993; Delvendahl et al.,  
172 2015; Silver et al., 1992). Therefore, we could use the dynamic clamp technique  
173 to implement the conductance of identical MF signals in inner- and outer-zone  
174 GCs based on the measured EPSC kinetics. We first applied input of a single MF  
175 with Poisson-distributed firing-frequencies ranging between 30 and 500 Hz for  
176 300 ms duration while changing the resting membrane potential to simulate the  
177 large variability of membrane potential of GCs observed *in vivo* (Chadderton et  
178 al., 2004). In line with the observed gradients in the electrophysiological  
179 properties of GCs, inner-zone GCs fired fewer action potentials compared with  
180 outer-zone GCs in response to low-frequency MF inputs at a membrane potential  
181 of  $\sim -90$  mV (**Figure 3B,C**). In contrast, inner-zone GCs fired more action  
182 potentials compared with outer-zone GCs in response to high-frequency MF  
183 inputs at a membrane potential of  $\sim -70$  mV. *In vivo*, such a depolarization would  
184 be caused by less inhibition and/or additional MF inputs. These data suggest that

185 outer- and inner-zone GCs are specialized for low- and high-frequency MF  
186 inputs, respectively.

### 187 **Fourier-like transformation of MF input frequency**

188 To further test whether inner- and outer-zone GCs can extract different frequency  
189 components from a MF input signal, which would resemble a Fourier-  
190 transformation, we varied the MF input frequency sinusoidally between 30 and  
191 300 Hz (**Figure 4A**). At a holding potential of -70 mV, inner-zone GCs responded  
192 preferentially to high-frequency MF inputs up to 300 Hz, while outer-zone GCs  
193 responded preferentially to low-frequency inputs up to 100 Hz. To estimate the  
194 optimal frequency at which inner- and outer-zone GCs preferentially fire action  
195 potentials, we calculated the phase angle (see Methods). The mean phase  
196 angle, at which GC preferentially fired, was  $145.9 \pm 10.4^\circ$  for inner-zone ( $n = 13$ )  
197 and  $102.5 \pm 18.3^\circ$  for outer-zone GCs ( $n = 9$ ) ( $P_{T-TEST} = 0.04$ ), representing an  
198 average firing frequency of 217 and 100 Hz for inner- and outer-zone GCs,  
199 respectively. Thus, the gradient in the biophysical properties enables the  
200 cerebellar GC layer to split incoming MF signals into different frequency bands  
201 and thereby to perform a Fourier-like transformation of the compound MF input  
202 signal.

### 203 **The position of PFs is correlated with the position of GC somata**

204 A Fourier-like transformation in the GC layer (i.e. a separation of the spectral  
205 components of MF signals) could be particularly relevant if downstream  
206 pathways are specialized for these spectral components. Early silver-stainings  
207 and drawings from Ramón y Cajal indicate that inner-zone GCs give rise to PFs  
208 close to the PC layer and outer-zone GCs give rise to PFs close to the pia  
209 (Eccles, 1967; Ramón y Cajal, 1911 but see Espinosa and Luo, 2008; Wilms and  
210 Häusser, 2015). To test this possibility, we examined the ascending and parallel  
211 branches of the GC axon. First, we investigated whether there is a correlation  
212 between the relative positions of the PF in the molecular layer and the GC  
213 somata in the GC layer. Dil was injected *in vivo* into the GC layer to label GCs  
214 and their axons. Several GCs were clearly stained 24h after Dil injection (**Figure**

215 **5A)**, and the position of their soma and PF in the cerebellar cortex could be  
216 measured (**Figure 5B-D**). Even though the length of the ascending GC axon  
217 showed considerable variation ( $196 \pm 5.5 \mu\text{m}$ , range: 144 to 291  $\mu\text{m}$ ,  $n = 39$   
218 axons in  $n = 6$  mice), after normalization for the thickness of the molecular and  
219 GC layers, GC soma position was significantly correlated with the position of the  
220 bifurcation in the GC axon (**Figure 5C,D**;  $R^2=0.86$ ,  $P<0.001$ ). These data show  
221 that inner- and outer-zone GCs preferentially give rise to inner- and outer-zone  
222 PFs, respectively.

### 223 **Inner-zone PFs have larger diameter and higher action potential** 224 **propagation velocity**

225 Next, we tested whether PFs, like GCs, have different properties depending on  
226 the position within the molecular layer. First, we compared the PF diameters in  
227 electron microscopic images of parasagittal sections of mouse cerebellum lobule  
228 V and found significantly larger diameters for inner-zone PFs compared to  
229 middle- and outer-zone PFs ( $182 \pm 2.6 \text{ nm}$ ,  $n = 703$  vs.  $159 \pm 2.0 \text{ nm}$ ,  $n = 819$  vs.  
230  $145 \pm 1.7 \text{ nm}$ ,  $n = 1085$  **Figure 6A-C**;  $P_{\text{ANOVA}} < 0.0001$ ), which is in agreement  
231 with previous investigations reported in cat (Eccles et al., 1967), monkey (Fox  
232 and Barnard, 1957), and rats (Pichitpornchai et al., 1994).

233 Axonal diameter is often a strong predictor for axonal conduction speed (Jack et  
234 al., 1983). We therefore recorded compound action potentials of PFs in lobule V  
235 and compared their conduction speed in the inner-, middle- and outer-zone of the  
236 molecular layer (**Figure 6D-F**). We detected a significantly higher velocity in  
237 inner-zone PFs compared with in middle- or outer-zone PFs ( $0.33 \pm 0.004 \text{ m s}^{-1}$ ,  
238  $n = 8$  vs.  $0.31 \pm 0.005 \text{ m s}^{-1}$ ,  $n = 6$  vs.  $0.28 \pm 0.005 \text{ m s}^{-1}$ ,  $n = 8$ ; **Figure 6F**;  
239  $P_{\text{ANOVA}} < 0.0001$ ). The absolute velocity and the gradient in the velocity from  
240 inner- to outer-zone PFs agree well with previous studies (Baginskis et al., 2009;  
241 Vranesic et al., 1994). These results suggest that the inner-zone PFs are  
242 specialized for fast signaling, which is consistent with the concept that inner-zone  
243 GCs are tuned for high-frequency inputs (cf. Figure 1 and 2).



244 In addition to the above results obtained from lobule V, similar gradients in both  
245 axon diameter and axon conduction speed were found in lobule IX (**Figure 6—**  
246 **figure supplement 1**). This suggests that gradients in axon diameter and axon  
247 conduction speed are general features of the cerebellar cortex.

248 A possible confounder of our results could be an overrepresentation of large-  
249 diameter Lugaro cell axons within inner-zone PFs (Dieudonne and Dumoulin,  
250 2000). However, this would predict that the histogram of the axon diameters  
251 shows two distinct peaks with varying amplitude. Instead, we observed a single  
252 bell-shaped distribution in each PF zone (**Figure 6—figure supplement 2**),  
253 arguing that the measured differences between axon diameters were not due to  
254 varying contributions from Lugaro cell axons, but reflect the differences between  
255 inner-, middle- and outer-zone PFs.

### 256 **PCs process inner-, middle-, and outer-zone PF inputs differentially**

257 Our data thus far indicate that GCs and PFs are adapted to different MF input  
258 frequencies and conduction velocity, respectively. This arrangement could in  
259 principle provide PFs with functionally segregated information streams that are  
260 differentially processed in PCs. To investigate this possibility, we made whole-  
261 cell current-clamp recordings from PCs in sagittal slices of the cerebellar vermis.  
262 PCs were held at a hyperpolarized voltage to prevent spiking and to isolate  
263 excitatory inputs. Electrical stimulation of the PFs was alternated between inner-,  
264 middle- and outer-zones and adjusted to obtain similar amplitude EPSPs in all  
265 zones (**Figure 7A,B**). Stimulation of inner-zone PFs resulted in EPSPs (Barbour,  
266 1993; Roth and Häusser, 2001) with shorter rise and decay times compared with  
267 EPSPs obtained from stimulating outer-zone PFs (rise<sub>20-80</sub>: inner:  $0.57 \pm 0.04$  ms,  
268  $n = 12$ ; middle:  $0.93 \pm 0.17$  ms,  $n = 4$ ; outer:  $1.83 \pm 0.33$  ms,  $n = 12$  ( $P_{ANOVA} =$   
269  $0.009$ ; decay: inner:  $21.9 \pm 1.5$  ms, middle:  $39.7 \pm 1.1$  ms outer:  $40.8 \pm 4.1$  ms;  
270  $P_{ANOVA} = 0.0004$ , **Figure 7C**). These results suggest that inner-zone PF inputs  
271 undergo less dendritic filtering in PCs compared with outer-zone PF inputs (De  
272 Schutter and Bower, 1994a, b; Roth and Häusser, 2001) but see (De Schutter  
273 and Bower, 1994c). To investigate high-frequency inputs to PCs, we elicited five

274 EPSPs at 100 Hz and 500 Hz (**Figure 7D,E**). Individual EPSPs evoked from  
275 inner-zone PFs showed clear individual rising phases and peaks between each  
276 stimulus and less summation compared with outer-zone PFs (**Figure 7D-F**).  
277 These results suggest that inner-zone PFs can transmit timing information more  
278 faithfully compared with outer-zone PFs and thus control spike timing of PCs  
279 more precisely.

### 280 **The observed neuronal gradients increase storing capacity and improve** 281 **temporal precision of PC spiking**

282 Thus far we have described a prominent gradient in the electrophysiological  
283 properties of GCs over the depth of the GC layer that enables inner- and outer-  
284 zone GCs to preferentially respond to high- and low-frequency inputs,  
285 respectively. The different frequency components are transferred via specialized  
286 PFs, which enable PCs to interpret high-frequency signals rapidly at the base of  
287 their dendritic trees and low-frequency signals slowly at more distal parts of their  
288 dendritic trees (**Figure 8A**).

289 To address the functional implications of these gradients in the GC and  
290 molecular layer, we performed computational modeling of a neuronal network of  
291 the cerebellar cortex with integrate-and-fire neurons. The model consisted of one  
292 PC and varying number of GCs and MFs (**Figure 8A**). GCs received randomly  
293 determined MF inputs with either tonic (Arenz et al., 2008; van Kan et al., 1993)  
294 or bursting (Rancz et al., 2007) *in vivo*-like spiking sequences. By changing the  
295 synaptic weights of the GC to PC synapses, the PC had to acquire a target  
296 spiking sequence with regular 80-, 40- and 120-Hz firing (**Figure 8B**). The  
297 algorithm for changing the synaptic weights was a combination of a learning  
298 algorithm based on climbing-fiber-like punishments and an unbiased  
299 minimization algorithm (see Methods).

300 We first compared a model without gradients, where the parameters were set at  
301 the average of the experimentally determined values, with a model including all  
302 experimentally determined gradients (black and red, respectively, throughout

303 **Figure 8**). To measure the difference between the final PC spiking and the target  
304 sequence we calculated van Rossum errors using a time constant of 30 ms  
305 (Rossum, 2001) **Figure 8C-E**). With increasing number of GCs, the final PC  
306 spiking sequence resembled the target sequences increasingly better, as  
307 illustrated by an average spiking histogram from many repetitions with different  
308 random sets of MF inputs for models consisting of 100 and 1000 GCs (**Figure**  
309 **8B**). As expected, the average minimal van Rossum error (for many repetitions  
310 with different random sets of MF inputs) decreased with increasing number of  
311 GCs (**Figure 8C**). For all sizes of the GC population, the average minimal van  
312 Rossum error was significantly smaller in the model containing all the  
313 experimentally determined gradients compared with the model without any  
314 gradients. For example, to obtain the spiking precision of the model containing  
315 400 GCs with all gradients, the model without gradients required 800 GCs (cf.  
316 red arrows in **Figure 8C**). This indicates that for a cerebellum exploiting gradients  
317 in the GC layer, the number of GCs can at least be halved to obtain a certain  
318 temporal precision compared with a cerebellum containing no gradients.

319 To investigate the relative contribution of each of the gradients, we tested models  
320 containing each gradient in isolation, resulting in intermediate van Rossum errors  
321 (blue, yellow, and green in **Figure 8C,D**). The average relative differences  
322 between the models across all sizes of the GC populations suggest an almost  
323 additive behavior of the individual gradients to the overall performance (**Figure**  
324 **8E**).

325 To further investigate the interplay of the different gradients, we investigated a  
326 model containing all gradients, but the connectivity between GCs, PF action  
327 potential speed, and PC EPSP kinetics were randomly intermixed (red dashed  
328 lines in Figure 8C-E). The network benefits from these intermixed gradients, but  
329 maximum optimization can only be obtained with correct connectivity (**Figure**  
330 **8E**).

331 The time constant of the van Rossum error can be decreased or increased to  
332 investigate spike timing or slower changes in firing rate, respectively. The impact  
333 of the gradients increased with increasing time constant (**Figure 8—figure**  
334 **supplement 1A,B**), indicating that rate coded signaling especially benefits from  
335 the here described gradients. To specifically test the effect of gradients on the  
336 cerebellum's ability to switch between firing frequencies, we made sigmoid fits  
337 around the times of firing rate changes. The transition time ( $t_T$ ; see methods)  
338 from these fits showed that models with all gradients showed on average 20%  
339 faster switching between firing frequencies than models without any gradients  
340 (**Figure 8—figure supplement 1C-F**).

341 To further test the influence of gradients on efficient cerebellar processing, we  
342 repeated the modeling experiments but used a target sequence with a firing  
343 pause (i.e. 80, 0, and 120 Hz instead of 80, 40, and 120 Hz) resulting in similar  
344 van Rossum errors and transition time (**Figure 8—figure supplement 1G-M**). A  
345 pause in firing enabled us to quantify the temporal error at the start and the end  
346 of the pause (**Figure 8—figure supplement 1N-Q**), because these spike times  
347 have been proposed to be of particular relevance for behavior (Hong et al.,  
348 2016). Both measures (transition time and temporal error in pause beginning and  
349 end) revealed similar results compared with the van Rossum measure, indicating  
350 that the speed of 'frequency-switching' and the temporal spiking precision of PCs  
351 critically depend on the here described gradients. Thus, our modeling results  
352 show that experimentally determined gradients improve the spiking precision,  
353 accelerate 'frequency-switching', and increase the storing capacity of the  
354 cerebellar cortex.

355

## 356 **Discussion**

357 In this study, we describe a gradient in the biophysical properties of superficial to  
358 deep GCs, which enables the GC layer to perform a Fourier-like transformation  
359 of the MF input. Furthermore, we show that the downstream pathways from GCs

360 to PCs are specialized for transmitting the frequency band for which the  
361 corresponding GCs are tuned to. Finally, computational modeling demonstrates  
362 that both the gradients in the GC layer and the specialized downstream pathways  
363 improve the spiking precision, accelerate the change of firing frequency of PCs,  
364 and increase storing capacity in the cerebellar cortex.

### 365 **Fourier-like transformation in the cerebellar cortex**

366 Our data demonstrate that outer-zone GCs preferentially fire during MF input with  
367 low frequency ('low-frequency' GCs, magenta in **Figure 9A**), whereas inner-zone  
368 GCs preferentially fire during MF input with high frequency ('high-frequency'  
369 GCs, green in **Figure 9A**). The separation of a signal into its frequency  
370 components resembles a Fourier transformation (**Figure 9B**). The analogy with a  
371 Fourier transformation has the limitations that (1) a single MF cannot transmit two  
372 frequencies simultaneously but only separated in time (cf. example in **Figure 9A**)  
373 and (2) concurrent inputs from two MFs with different frequencies synapsing onto  
374 a single GC cannot be separated. Yet, our data indicate that the entire GC layer  
375 with several MFs sending various frequencies to numerous GCs can execute a  
376 Fourier-like transformation. In analogy to the dispersion of white light in an optical  
377 prism into its spectral components, the broadband MF signal is separated into its  
378 spectral components with inner- to outer-zone GCs preferentially transmitting the  
379 high- to low frequency components, respectively. Such a separation offers the  
380 chance to process each frequency component differentially. Indeed, in the  
381 molecular layer, the high-frequency components of the MF signal are sent via  
382 rapidly conducting axons to proximal parts of the PC dendritic tree. This allows  
383 fast (phasic) signals to have a strong and rapid impact on PC firing. On the other  
384 hand, low-frequency components of the MF signal are conducted more slowly  
385 and elicit slower EPSPs, allowing slow (tonic) signals to have a modulatory  
386 impact on PC firing. Our data indicate that, in analogy to the increased storing  
387 capacity of digital audio and image compression (Jayant et al., 1993; Wallace,  
388 1992), the combination of a Fourier-like transformation in the GC layer and  
389 specialized downstream signaling pathways in the molecular layer dramatically  
390 reduces the number of required GCs for precise PC spiking (cf. **Figure 8**).

391 Furthermore, our data support the ‘adaptive filter’ theory of the cerebellum, where  
392 broadband MF input is differentially filtered by GCs (Dean et al., 2010; Fujita,  
393 1982; Singla et al., 2017). Within this framework, our data indicate a gradient in  
394 the band-pass filtering properties of GCs. Furthermore, our data could provide an  
395 additional explanation for improved motor learning by elevated background  
396 activity of MFs (Albergaria et al., 2018), because the elevated MF activity will  
397 help overcoming the high threshold of inner-zone GCs, which rapidly and  
398 effectively impact PCs via fast conducting PFs at the proximal dendrite.

### 399 **Axes of frequency specialization in the cerebellum**

400 There are at least two axes of heterogeneity in the cerebellar cortex. First, Zebrin  
401 stripes can be observed as parasagittal zones (‘medio-lateral’ axis) in cerebellar  
402 cortex (Apps et al., 2018). Firing rate, firing regularity, synaptic connectivity and  
403 even synaptic plasticity seems to differ between PCs in zebrin positive and  
404 negative zones (Valera et al., 2016; Wadiche and Jahr, 2005; Xiao et al., 2014;  
405 Zhou et al., 2014). Second, there is a lobular organization (‘rostro-caudal’ axis)  
406 as shown here by the functional differences between lobules V and IX (**Figure**  
407 **1—figure supplement 1**). GCs in lobule IX are tuned to lower frequencies than  
408 GCs in lobule V. These findings are largely in line with previous investigations  
409 (Heath et al., 2014; Witter and De Zeeuw, 2015a; Zhou et al., 2014), where the  
410 anterior cerebellum was identified to process high-frequency or bursting signals,  
411 while the vestibulo-cerebellum mainly processed lower frequency or slowly-  
412 modulating inputs. Furthermore, the optimal time intervals for introduction of  
413 spike timing dependent plasticity differ between the vermis and the flocculus  
414 (Suvrathan et al., 2016).

415 In addition to these two known axes of heterogeneity, we described an axis that  
416 is orthogonal to the surface of the cerebellar cortex. This ‘depth’ axis causes  
417 inner-zone GCs to be tuned to higher frequencies than outer-zone GCs. This  
418 frequency gradient along the ‘depth’-axes is in line with recently described  
419 connections of MFs and PC, which specifically target GCs close to the PC layer  
420 (Gao et al., 2016; Guo et al., 2016). These connections send slow feedback

421 signals to the outer-zone GCs, which — according to our framework — are  
422 ideally suited to process such slow modulatory signals. Independent of these  
423 specialized feedback pathways, MFs exhibit heterogeneity (Chabrol et al., 2015;  
424 Bengtsson and Jörntell, 2009). Our data indicate that each type of the  
425 heterogeneous MF inputs is split into its frequency components along the depth  
426 axis. Our results furthermore predict that superficial GCs, such as the ones  
427 imaged recently in the investigation of eye-blink conditioning and reward  
428 representation in the cerebellar cortex (Giovannucci et al., 2017; Wagner et al.,  
429 2017), would preferentially convey low-frequency signals to PCs and might not  
430 be representative for the full range of frequencies present over the depth of the  
431 GC layer.

432 Thus, including this new ‘depth’ axis, there are three orthogonal axes along  
433 which the cerebellar cortex is tuned for preferred frequency, indicating the  
434 importance of proper frequency tuning of the circuitry.

#### 435 **The role of inhibition**

436 In the current study we did not investigate molecular layer interneurons, which  
437 can have large impact on PC spiking (Blot et al., 2016; Dizon and Khodakhah,  
438 2011; Gaffield and Christie, 2017; Mittmann et al., 2005; Sudhakar et al., 2017).  
439 However, the spatial arrangement of stellate and basket cell interneurons is  
440 consistent with our framework. Although the dendrites of molecular layer  
441 interneurons can span the entire molecular layer, the dendrites of basket cells  
442 seem to be preferentially located at the inner-zone of the molecular layer  
443 (Palkovits et al., 1971; Rakic, 1972), which positions them ideally to receive rapid  
444 high-frequency signals of inner-zone PFs. Consistently, they impact PC firing  
445 rapidly and efficiently via their pinceaus (Blot and Barbour, 2014). Furthermore,  
446 the dendrites of a subset of stellate cells (with their somata located in the outer-  
447 zone molecular layer) are preferentially located at the outer-zone molecular layer  
448 (Palkovits et al., 1971; Rakic, 1972), which positions them ideally to receive  
449 modulatory low-frequency signals and elicit slow IPSPs in PCs. Furthermore,  
450 molecular layer interneurons seem to represent a continuum along the vertical

451 axis, with a correlation between the vertical location of the soma, axonal boutons,  
452 and dendrite location (Sultan and Bower, 1998), which is consistent with the  
453 here-described continuum of biophysical properties along the vertical axis of the  
454 cortex. Incorporating molecular layer interneurons, their synaptic plasticity and  
455 their potential gradients into the frequency-dispersion framework may show a  
456 further increase in the dynamic range of frequency separation within the  
457 cerebellar cortex over what we have described here (Gao et al., 2012).

#### 458 **Functional implications for the cerebellum**

459 In general, our anatomical and electrophysiological data, combined with our  
460 modeling results, show that inner-zone GCs convey high-frequency signals from  
461 MFs via rapid pathways to PCs with little filtering. This is in contrast to outer-zone  
462 GCs that are tuned to lower frequencies, and which signals undergo more  
463 filtering in the PC. These results suggest that the sparse code of GCs (Albus,  
464 1971; Billings et al., 2014; Marr, 1969) is in part caused by different frequency  
465 tuning of GCs.

466 MF firing frequencies range from  $<1$  to  $\sim 1000$  Hz (Arenz et al., 2008; Chadderton  
467 et al., 2004; Jörntell and Ekerot, 2006; Rancz et al., 2007; van Kan et al., 1993).  
468 Many previous modeling studies investigating cerebellar function considered the  
469 activity of each MF as a constant digital value (Albus, 1971; Babadi and  
470 Sompolinsky, 2014; Brunel et al., 2004; Clopath et al., 2012; Marr, 1969), a  
471 constant analog value (Chabrol et al., 2015; Clopath and Brunel, 2013), or spike  
472 sequences with constant frequency (Billings et al., 2014; Cayco-Gajic et al.,  
473 2017; Steuber et al., 2007). We focused on the time-varying aspects of MF  
474 integration in GCs, and therefore implemented a model with a corresponding  
475 large range of MF input frequencies that could change over time. It would be  
476 interesting to elucidate in, as much previous models, consisting of more uniform  
477 MF inputs, would benefit from the here-observed biophysical gradients.

478 To implement these gradients in a model we used a simplified cerebellar circuitry  
479 that does not consider active dendrites (Llinás and Sugimori, 1980) or the tonic



480 activity of PCs (Raman and Bean, 1997). It will therefore be interesting to  
481 investigate if the here-observed gradients in the GC and molecular layer improve  
482 the performance of more complex models of the cerebellar cortex (De Schutter  
483 and Bower, 1994a; Garrido et al., 2013; Masoli et al., 2015; Medina et al., 2000;  
484 Rossert et al., 2015; Spanne and Jörntell, 2013; Steuber et al., 2007; Sudhakar  
485 et al., 2017; Walter and Khodakhah, 2009; Yamazaki and Tanaka, 2007).  
486 Furthermore, it remains to be investigated whether gradients in the GC layer also  
487 improve models that aim to explain tasks such as eye-blink conditioning (Mauk  
488 and Buonomano, 2004) and vestibulo-ocular reflexes (du Lac et al., 1995)

489 Our model simulated the learning that PCs undergo to acquire specific firing  
490 frequencies in response to GC input. PC firing rate and spiking precision have  
491 been shown to be closely related to movement (Brown and Raman, 2018;  
492 Sarnaik and Raman, 2018). Our results show that the same temporal spiking  
493 precision or the same frequency switching speed can be obtained with  
494 approximately half the number of GCs when GC gradients are implemented  
495 (**Figure 8**). Taking into account the large number of cerebellar GCs in the brain  
496 (Herculano-Houzel, 2009; Williams and Herrup, 1988), a significant reduction in  
497 the number of GCs could represent an evolutionary advantage to minimize  
498 neuronal maintenance energy (Howarth et al., 2012; Isler and van Schaik, 2006).  
499 Therefore, the dramatic increase in storing capacity for precise PC spiking  
500 provides an evolutionary explanation for the emergence of gradients in the  
501 neuronal properties.

## 502 **Functional implications for other neural networks**

503 Based on the described advantages of the Fourier transformation for rapid and  
504 storing-efficient information processing, we hypothesize that other neural  
505 networks also perform Fourier-like transformations and use segregated  
506 frequency-specific signaling pathways. To our knowledge this has rarely been  
507 shown explicitly, but similar mechanisms might operate, for example, in the  
508 spinal cord network: descending motor commands from the pyramidal tract send  
509 broadband signals to motoneurons with different input resistances resulting from

510 differences in size. This enables small motoneurons to fire during low-frequency  
511 inputs and large motoneurons only during high-frequency inputs (Henneman et  
512 al., 1965). Furthermore, specialized efferent down-stream signaling pathways  
513 innervate specific types of muscles with specialized short-term plasticity of the  
514 corresponding neuromuscular junctions (Wang and Brehm, 2017).

515 In the hippocampus, frequency preferences of hippocampal neurons are well  
516 established enabling segregation of compound oscillatory input into distinct  
517 frequency components (Pike et al., 2000). Furthermore, there is increasing  
518 evidence that previously considered homogeneous population of neurons exhibit  
519 gradients in the neuronal properties (Cembrowski and Spruston, 2019), such as  
520 the intrinsic electrical properties and synaptic connectivity in CA3 pyramidal  
521 neurons (Galliano et al., 2013). The heterogeneity furthermore enables functional  
522 segregation of information streams for example in CA1 pyramidal neurons  
523 (Soltesz and Losonczy, 2018). Finally, in the neocortex, gradients in anatomical  
524 and biophysical properties were recently uncovered (Fletcher and Williams,  
525 2019).

526 In summary, our findings contribute to the growing body of evidence that the  
527 neurons of a cell layer can exhibit systematic functional heterogeneities with  
528 differential tuning of neurons along gradients. Our data furthermore suggest that  
529 such gradients facilitate complex transformation of information, such as Fourier-  
530 like transformations, to cope with a broad temporal diversity of signals in the  
531 central nervous system.

532

## 533 **Material and Methods**

### 534 **Electrophysiology**

535 Parasagittal 300- $\mu$ m thick cerebellar slices were prepared from P21–P30 (young  
536 animals) or from P80-P100 (old animals) C57BL/6 mice of either sex as  
537 described previously (Ritzau-Jost et al., 2014);(Delvendahl et al., 2015). Animals  
538 were treated in accordance with the German and French Protection of Animals

539 Act and with the guidelines for the welfare of experimental animals issued by the  
540 European Communities Council Directive. The extracellular solution for the  
541 whole-cell measurements contained (in mM): NaCl 125, NaHCO<sub>3</sub> 25, glucose 20,  
542 KCl 2.5, CaCl<sub>2</sub> 2, NaH<sub>2</sub>PO<sub>4</sub> 1.25, MgCl<sub>2</sub> 1 (310 mOsm, pH 7.3 when bubbled with  
543 Carbogen (95%O<sub>2</sub>/5%CO<sub>2</sub>)). For outside-out measurements of potassium  
544 currents (Figure 2), 150 μM CdCl<sub>2</sub> and 1 μM TTX were added to the external  
545 solution to block voltage-gated calcium channels and sodium channels,  
546 respectively. The intracellular solution contained in mM: K-Gluconate 150, NaCl  
547 10, K-Hepes 10, Mg-ATP 3, Na-GTP 0.3, EGTA 0.05 (305 mOsm, pH 7.3). A  
548 liquid junction potential of +13 mV was corrected for. All electrophysiological  
549 measurements were performed with a HEKA EPC10 amplifier (HEKA Elektronik,  
550 Lambrecht/Pfalz, Germany) under control of the Patchmaster software. All  
551 measurements were performed at 34–37°C.

552

#### 553 *Current clamp recordings in GCs*

554 Action potentials were evoked in current-clamp mode by current pulses  
555 (amplitude 20–400 pA, duration 300 ms). To determine the input resistance,  
556 subthreshold current pulses were applied from -20 to + 20 pA in 2 pA steps. The  
557 resistance of the solution-filled patch-pipettes was between 6–12 MΩ and the  
558 mean series resistance was not compensated during the measurement. Data  
559 were sampled at 200 kHz.

560

#### 561 *Outside-out recordings in GCs*

562 To reliably clamp potassium currents from the soma of GCs (**Figure 2**),  
563 potassium currents were measured in outside-out patches pulled from the soma  
564 of inner and outer GCs by applying 10 ms voltage steps from -90 to +60 mV with  
565 10 mV increments at an intersweep interval of 1 s. The intersweep holding  
566 potential was -90 mV. Data were sampled at 100 kHz.

567

#### 568 *Compound action potentials in PFs*

569 For the detection of compound action potentials in PFs, two pipettes (tip

570 resistances 1–4 M $\Omega$ ) filled with extracellular solution and connected to the patch-  
571 clamp amplifier were positioned within the molecular layer of horizontally cut  
572 slices of the cerebellar vermis. The average distance between two recording  
573 electrodes was  $143 \pm 5 \mu\text{m}$ . Compound action potentials were evoked by voltage  
574 stimulation (100 V) for 100  $\mu\text{s}$  with a third pipette connected to an accumulator  
575 powered stimulation device (ISO-Pulser ISOP1, AD-Elektronik, Buchenbach,  
576 Germany). 40 to 80 stimulations delivered at 1 Hz were averaged and analyzed.

577

#### 578 *Excitatory postsynaptic potentials in PC*

579 Excitatory postsynaptic potentials (EPSPs) in PC were elicited by voltage  
580 stimulation of the PFs within the inner, middle or outer third of the molecular layer  
581 from horizontally cut cerebellar slices (Figure 7). 10  $\mu\text{M}$  SR95531 was added to  
582 the external solution to block GABA<sub>A</sub> receptors. The stimulation pipette was filled  
583 with extracellular solution, and the voltage was adjusted between 6 to 25 V to  
584 elicit EPSPs with an amplitude between 1 and 2 mV. EPSPs were measured  
585 after a single 100  $\mu\text{s}$  voltage stimulation or 5 stimulations (100  $\mu\text{s}$  duration) at a  
586 frequency of 100 and 500 Hz. Averages of 30 trains per stimulation protocol were  
587 used for data analysis.

588

#### 589 *Excitatory postsynaptic currents in GCs*

590 To measure evoked EPSCs from GCs (**Figure 3—figure supplement 1**), 90-100  
591 days-old mice were used. GCs from inner- or outer-zone from lobule V were held  
592 at resting conditions and MF axons were stimulated at 1 Hz with a second  
593 pipette. The average stimulation voltage was  $36 \pm 3 \text{ V}$  for outer-zone GCs and  $37$   
594  $\pm 3 \text{ V}$  for inner-zone GCs.

595

#### 596 **Dynamic Clamp of MF conductance in GCs**

597 In order to analyze the response of GCs on *in-vivo* like MF inputs, we used a  
598 dynamic clamp implemented with the microcontroller Teensy 3.6  
599 (<https://www.pjrc.com>) as described by Desai et al. (Desai et al., 2017). The  
600 Teensy was programmed using the Arduino integrated development environment

601 with the code provided by Desai et al (2017) and modified for our need as  
602 described in the following.

603

604 The time course of MF conductance was

605 
$$G_{EPSC}(t) = G_{max} A_{norm} \left( -e^{-\frac{t}{\tau_r}} + \sum_{i=1}^3 a_i e^{-\frac{t}{\tau_i}} \right)$$

606 where the exponential rise time ( $\tau_r$ ) was 0.1 ms, the decay time constants ( $\tau_1$ ,  $\tau_2$ ,  
607 and  $\tau_3$ ) were 0.3, 8, and 40 ms, respectively, and the relative amplitude of the  
608 decay components ( $a_1$ ,  $a_2$ , and  $a_3$ ) were 0.7, 0.26, and 0.04, respectively. The  
609 peak conductance ( $G_{max}$ ) was 1 nS (Hallermann et al., 2010) and the  
610 normalization factor ( $A_{norm}$ ) was 0.518, which was numerically calculated to  
611 obtain a peak amplitude of 1. The kinetics of the MF conductance were chosen to  
612 reproduce the measured mixed AMPA and NMDA EPSC kinetics of single  
613 EPSCs (**Figure 3—figure supplement 1**) and trains of EPSCs (Baade et al.,  
614 2016). The short-term plasticity during Poisson sequence of spikes was  
615 implemented by changing  $G_{max}$  according to a simple phenomenological model  
616 (Tsodyks and Markram, 1997) assuming a release probability  $p_{r0}$  of 0.4 (Ritzau-  
617 Jost et al., 2014). Facilitation was implemented as an increase in the release  
618 probability according to  $p_r = p_r + 0.2*(1 - p_r)$  and decaying back to  $p_{r0}$  with a time  
619 constant of 12 ms (Saviane and Silver, 2006). Depression was implemented  
620 according to a recovery process with a time constant of 25 ms, which  
621 approximates a biexponential recovery process of 12 ms and 2 s (Hallermann et  
622 al., 2010; Saviane and Silver, 2006). The resulting short-term plasticity  
623 reproduced previously obtained data with regular spiking ranging from 20 to 1000  
624 Hz (Baade et al., 2016; Hallermann et al., 2010; Ritzau-Jost et al., 2014).

625

626 The microcontroller was programmed to implement the MF conductance and its  
627 short-term plasticity with Poisson distributed spike times with a constant  
628 frequency ranging from 30 to 500 Hz for 300 ms (Figure 3). In each cell, each  
629 frequency was applied five times.

630

631 To investigate the response to sinusoidally varying input frequencies (Figure 4),  
632 the target frequency of the Poisson process ( $F$ ) was varied on a logarithmic scale  
633 according to:

$$634 \quad F(t) = \exp\left(\log(F_{min}) + (\log(F_{max}) - \log(F_{min}))\left(0.5 + 0.5\sin(2\pi t/T)\right)\right)$$

635 where the minimal and maximal frequency ( $F_{min}$  and  $F_{max}$ ) were 30 and 300 Hz,  
636 respectively, and the duration of the sine wave cycle ( $T$ ) was 1 s. In each cell, 10  
637 cycles were applied consecutively. The histogram of the spike times (Figure 4B)  
638 was averaged across the last six cycles of all cells. The vector strength and  
639 phase angle (Kan et al., 1993) were calculated as the absolute value and the  
640 argument of the complex number  $\rho$  ( $i = \sqrt{-1}$ ):

$$641 \quad \rho = \frac{1}{N} \sum_{n=1}^N e^{i2\pi \frac{t_n}{T}}$$

642 where  $t_n$  are the spike times of all  $N$  spikes per experiment and  $T$  the cycle  
643 duration (1 s).

## 645 **Electron Microscopy**

646 Four C57BL/6 mice of either sex with an age between P23–P28 were sacrificed,  
647 followed by transcardial perfusion with saline and consecutively a fixative  
648 containing 4% paraformaldehyde and 2% glutaraldehyde in phosphate-buffered  
649 saline (PBS). After removal of the brain, the tissue was allowed to post-fix over  
650 night at 4°C and sagittal sections of the cerebellum were prepared at a thickness  
651 of 60  $\mu\text{m}$  using a Leica microtome (Leica Microsystems, Wetzlar, Germany). The  
652 sections were stained in 0.5% osmium tetroxide in PBS for 30 min followed by  
653 dehydration in graded alcohol and another staining step with 1% uranyl acetate  
654 in 70% ethanol. After further dehydration, the tissue was embedded in durcupan  
655 (Sigma-Aldrich), which was allowed to polymerize for 48 h at 56°C between  
656 coated microscope slides and cover glasses. Regions of interest were identified  
657 by light microscopy, cut and transferred onto blocks of durcupan to obtain ultra-

658 thin sections using an Ultramicrotome (Leica Microsystems). Ultra-thin sections  
659 were transferred onto formvar-coated copper grids and stained with lead citrate.  
660 Ultrastructural analysis was performed using a Zeiss SIGMA electron microscope  
661 (Zeiss NTS, Oberkochen, Germany) equipped with a STEM detector and ATLAS  
662 software.

663

#### 664 **Measurement of parallel-fiber axon diameter**

665 Electron micrographs were manually analyzed in a blind manner (numbered by  
666 masked randomization) and each micrograph was divided into eight identically  
667 sized fields. The diameter of each parallel-fiber axon was measured as the  
668 longest chord in one or two of these fields. Cross sections with visible active  
669 zones or mitochondria were excluded from analysis.

670

#### 671 **Dil Injections and GC tracking**

672 Six P20 CD1 mice were anesthetized with isoflurane (4%). An incision of the skin  
673 to expose the skull and a hole was manually drilled using a 25G needle above  
674 the desired injection site. Injections of small amounts of Dil (1,1-dioctadecyl-  
675 3,3,3,3 tetramethylindocarbocyanine perchlorate, ThermoFisher Scientific, 10%  
676 in N,N-dimethylformamide) were performed using a broken glass pipette  
677 connected to a picospritzer II (Parker Instrumentation). 24 h after injection,  
678 animals were sacrificed and transcardially perfused with 4% paraformaldehyde in  
679 PBS. The cerebellum was dissected, fixed overnight, and embedded in 4%  
680 agarose in PBS. 150- $\mu$ m thick sections were then cut in the transverse or sagittal  
681 plane using a vibratome (VT1000, Leica microsystems). Z-Stacks (1  $\mu$ m steps)  
682 were acquired using a confocal microscope (Leica SP5 II, 63x objective). GCs  
683 were traced from their soma to the axonal bifurcation of PFs. (Average stack  
684 depth:  $84 \pm 20$   $\mu$ m). GC axons were reconstructed using the 'Simple Neurite  
685 Tracer' plugin (Longair et al., 2011) in Fiji (ImageJ, NIH, USA). This plugin  
686 allowed us to assess the continuity of axons between several cross-sections. GC  
687 ascending axons were then fully traced and measured within the Z-limits of  
688 image sections. The size of the different layers of cerebellar cortex was

689 reconstructed in each Z-stack. To avoid variability, all distances were normalized  
690 to the corresponding molecular layer height.

691

## 692 **Data analysis**

693 Data were analyzed using custom-written procedures in Igor Pro software  
694 (WaveMetrics, Oregon, USA). Intrinsic properties of GCs were determined from  
695 the injected currents that elicited the largest number of action potentials. The  
696 action potential threshold was defined as the membrane voltage at which the first  
697 derivative exceeded  $100 \text{ V s}^{-1}$ , the minimal action potential peak was set as -20  
698 mV and the minimal amplitude to 20 mV. All action potentials with a half-width  
699 smaller than  $50 \mu\text{s}$  and larger than  $500 \mu\text{s}$  were excluded. Action potential  
700 frequency and half-width were calculated from the first three action potentials.  
701 Membrane capacitance, resting membrane potential and series resistance were  
702 read from the amplifier software (HEKA) after achieving the whole-cell  
703 configuration. Input resistance ( $R_{in}$ ) was analyzed from alternating subthreshold  
704 current injections from -20 to 20 pA (2 pA steps). The voltage was plotted against  
705 injected current and a spline interpolation was performed to obtain the slope at  
706 the holding membrane potential (0 pA current injection).

707 Peak-current from outside-out patches was determined from voltage steps (-90  
708 to +60 mV) with Fitmaster software (HEKA). Steady-state inactivation was  
709 determined from the last 2 ms of the respective sweep. Cells were only included  
710 if  $50 \text{ pA} < I_{max} < 1 \text{ nA}$  to exclude potential whole-cell measurements and  
711 membrane-vesicles.

712

713 EPSP measurements from PCs and EPSC measurements from GCs were  
714 analyzed with the Fitmaster software (HEKA). For PC EPSPs, 20-80% rise time  
715 and time to peak were determined from the average of 30 individual single  
716 EPSPs. GCs EPSCs were averaged from 25 traces. To obtain the decay  
717 kinetics, single EPSPs/EPSCs were fitted with either one or two exponentials.  
718 The weighted time constant was calculated as:



719 
$$\tau_w = \frac{A_{\text{slow}} \tau_{\text{slow}} + A_{\text{fast}} \tau_{\text{fast}}}{A_{\text{slow}} + A_{\text{fast}}}$$

720 Paired-pulse ratio was determined between the first and the 5<sup>th</sup> EPSP after  
721 stimulation with 100 Hz trains. Single EPSCs from inner- and outer-zone GCs  
722 were averaged and fitted with two exponentials. The decay kinetics and  
723 amplitude of the grand-average was used to implement the MF EPSCs for the  
724 Dynamic Clamp.

725

## 726 **Neuronal networking modeling**

727 The neuronal network consisted of varying numbers of MF inputs, GCs and one  
728 PC and was implemented in Matlab (The MathWorks, Inc, Natick,  
729 Massachusetts, R2017a). For each simulation, a random set of MF inputs was  
730 generated. This input was then fed to a layer of integrate-and-fire GCs. A  
731 modeled PC then used the output of these GCs for trial-to-trial learning. The PF-  
732 to-PC synaptic weights were optimized with the aim to make the PC spiking  
733 sequence similar to the target sequence. In the following, each component of the  
734 model is explained in detail.

735

### 736 *MF inputs*

737 To simulate *in vivo* like MF firing patterns, half of the MFs fired tonically (van Kan  
738 et al., 1993) and the other half fired bursts (Rancz et al., 2007). All MF spike  
739 trains were modeled first by generating a ‘threshold trace’. For tonically firing  
740 MFs, this threshold trace was a Gaussian function with a peak and standard  
741 deviation chosen from uniform distributions ranging between 10 and 100 Hz and  
742 0.2 and 0.5 s respectively, and a peak time point between 0 and 0.5 s. For burst  
743 firing MFs, the threshold trace was an exponential function with a peak randomly  
744 chosen between 600 and 1200 Hz, a decay time constant of 30 ms and a peak  
745 between 0 and 0.5 s. The threshold trace was then evaluated against random  
746 numbers from a uniform distribution to determine the occurrence of a spike. To  
747 accelerate the simulations the sampling time interval was 1 ms.

748

749 *GC properties*

750 GCs were implemented as integrate and fire models with the following  
751 parameters: the membrane resistance was linearly varied between 450 M $\Omega$  for  
752 inner GCs to 800 M $\Omega$  for outer GCs (Figure 1H) and the threshold was linearly  
753 varied between -37 mV for inner GCs to -42 mV for outer GCs (Figure 1G). For  
754 the models without the GC gradient, these values were set to the mean of the  
755 values for the inner and outer GC (i.e. 625 M $\Omega$  and -39 mV). The reset potential  
756 was set to -90 mV and the membrane potential to -80 mV.

757

758 *PF properties*

759 To simulate a different action potential propagation speed, the GC spike times  
760 were delayed by a value linearly varied between 0 for inner and 3 ms for outer  
761 GCs. The delay was calculated as the difference in conduction time required to  
762 travel 5 mm with a speed of 0.28 and 0.33 m s<sup>-1</sup> (Figure 4F). Even with this  
763 anatomically rather too large PF length (Harvey and Napper, 1991), the PF  
764 propagation speed has only a small impact on the model performance (see e.g.  
765 blue lines and bars in Figure 7C-E), arguing against a big impact of PF  
766 conduction delays (Braitenberg et al., 1997) at least in our model approach.

767

768 *Synaptic connections and properties*

769 Each MF was connected to 10 GCs and each GC received 2 MF inputs, i.e. the  
770 number of MF was 1/5 of the number of GCs. Since our model consists only of  
771 'active' MFs, we chose only 2 and not 4 MFs per GCs (Billings et al., 2014). The  
772 MF to GC synapse was implemented as a model with one pool of vesicles with a  
773 release probability of 0.5 and a vesicle recruitment time constant of 13 ms  
774 (Hallermann et al., 2010). Synaptic facilitation was implemented by increasing  
775 the release probability after each spike by 0.2 decaying to the resting release  
776 probability with a time constant of 12 ms (Saviane and Silver, 2006). The  
777 synaptic conductance had exponential rise and decay time constants of 0.1 and  
778 2 ms, respectively, and a peak amplitude of 1.9 nS (Silver et al., 1992).  
779 Correspondingly, the GC to PC synapse was implemented as a model with one

780 pool of vesicles with a release probability ( $p_{r0}$ ) of 0.4 and a vesicle recruitment  
781 time constant of 50 ms. Synaptic facilitation was implemented by increasing the  
782 release probability after each spike by 0.2 decaying to the resting release  
783 probability with a time constant of 50 ms (Doussau et al., 2017; Isope and  
784 Barbour, 2002; Valera et al., 2012). The synaptic conductance had exponential  
785 rise time constant between 0.5 and 2 ms and decay time constant between 17.5  
786 and 70 ms for inner- and outer-zone GCs, respectively (Figure 6). The peak  
787 amplitude was adjusted to equalize the charge of the EPSC and to generate an  
788 approximately correct number of PC spikes (with the initial start values, i.e. all  
789 GC to PC synaptic weight factors = 1) by linearly varying between 0.5 and 0.15  
790 nS for inner- and outer-zone GCs, respectively.

791

#### 792 *PC properties*

793 The PC was implemented as an integrate and fire model with a membrane  
794 resistance of 15 M $\Omega$ , resting membrane potential of -50 mV, and a firing  
795 threshold of -45 mV. Spontaneous firing of PCs (Raman and Bean, 1997) was  
796 not implemented, and the only inputs to drive PCs to threshold were the GC-to-  
797 PC EPSCs.

798

#### 799 *Target sequence and van Rossum measure*

800 Based on *in vivo* firing patterns (Witter and De Zeeuw, 2015a), an arbitrary target  
801 firing sequence of 80, 40, and 120 Hz for 300, 100, and 100 ms, respectively,  
802 was chosen. The distance between the PC and the target spiking sequence was  
803 quantified with the van Rossum error (van Rossum, 2001). Both spiking  
804 sequences were convolved with an exponential kernel with a decay time constant  
805 of 30 ms (or values ranging from 2 to 300 ms in **(Figure 8—figure supplement**  
806 **1A,B)**). The van Rossum error was defined as the integral of the square of the  
807 difference between these two convolved traces. We also tested another  
808 algorithm to calculate the van Rossum error (Houghton and Kreuz, 2012), which  
809 C++ code was taken from <http://pymuvr.readthedocs.io/> and incorporated into  
810 Matlab via the MEX function and results were comparable.

811

812 *Learning and minimization algorithm*

813 For each random set of MF inputs, the GC to PC synaptic weights were changed  
814 according to the following algorithm with the aim to minimize the van Rossum  
815 error between the PC spiking sequence and the target sequence. The initial  
816 values of the synaptic weights were 1, and values were allowed to change  
817 between 0 and 100. First, an algorithm was used that was based on supervised  
818 learning (Raymond and Medina, 2018) to punish the GCs that have spikes that  
819 precede unwanted PC spikes. Subsequently, an unbiased optimization of the GC  
820 to PC synaptic weight was performed using the `patternsearch()` algorithm of  
821 Matlab to minimize the van Rossum error. To increase the chance that a global  
822 (and not local) minimum was found, the minimization of the routine was repeated  
823 several times with random starting values. Other optimization routines such as a  
824 simplex [`fminsearch()` of Matlab] or a genetic algorithm (`ga()` of Matlab)] revealed  
825 similar results. To exclude the possibility that the differences in the minimal van  
826 Rossum error between models with and without gradients were due to a bias in  
827 our learning algorithm, we performed a set of simulations with networks  
828 consisting of less than 100 GCs, in which we skipped the learning algorithm and  
829 only used unbiased minimization algorithm. This resulted in similar difference in  
830 the minimal van Rossum error between models with and without gradients,  
831 indicating that the learning algorithm was not biased towards one type of model.  
832 For networks with consisting of more than 100 GCs the pre-learning was required  
833 to facilitate the finding of the global minimum.

834

835 *Analysis of modeling results*

836 300 different sets of random MF inputs were used to determine 300 statistically  
837 independent minimal van Rossum values for each of the models with different  
838 number of GCs and different number of implemented gradients (illustrated as  
839 mean  $\pm$  SEM in Figure 7C). Comparing different models with the same set of MF  
840 input (using the nonparametric paired Wilcoxon signed-rank statistical test) the  
841 difference was significant ( $p < 0.001$ ) for all of the models and all number of GCs.

842 The van Rossum errors were then normalized to the mean of the error of the  
843 model without gradients (Figure 7D). The values in **Figure 8D** were fitted with  
844 cubic spline interpolation using the logarithm of the number of GCs as abscissa.

845

846 To quantify the transition time between two target frequencies of the PC, the  
847 spike histogram was fitted with the equation

848 
$$f(t) = 80 + \frac{-80 + 40}{1 + e^{-(t-200)/t_T}} + \frac{-40 + 120}{1 + e^{-(t-300)/t_T}}$$

849 where  $f$  is the spike frequency in Hz and  $t$  the time in ms. The transition time  $t_T$   
850 corresponds to the 23% to 77% decay and rise time for the transition from 80 to  
851 40 Hz and from 40 to 120 Hz, respectively.

852

### 853 *Sensitivity of model parameters*

854 We verified that our conclusions do not critically depend on specific parameters  
855 of the model. For example, decreasing the simulation time interval from 1 ms to  
856 100  $\mu$ s, resulted in difference of the best van Rossum error of 21% between  
857 models with and without gradients consisting of 100 GC, compared with a  
858 difference of 17% between the corresponding models with the default simulation  
859 time interval of 1 ms (cf. **Figure 8D**). With 4 MFs per GCs (not 2) the difference  
860 of the best van Rossum error was 15% between models with and without  
861 gradients consisting of 100 GC (17% with 2 MF per GC). With a membrane  
862 resistance of the PC of 100 M $\Omega$  (not 15 M $\Omega$ ) the difference of the best van  
863 Rossum error was 23% between models with and without gradients consisting of  
864 100 GC (17% with 15 M $\Omega$ ). Finally, changing the target sequence to 80, 0, and  
865 120 Hz (not 80, 40, and 120 Hz) resulted in very similar results as obtained with  
866 the original target (compare Figure **Figure 8C-E** with **Figure 8—figure**  
867 **supplement 1 H-J** and **Figure 8—figure supplement 1 D-F** with **Figure 8—**  
868 **figure supplement 1 K-M**).

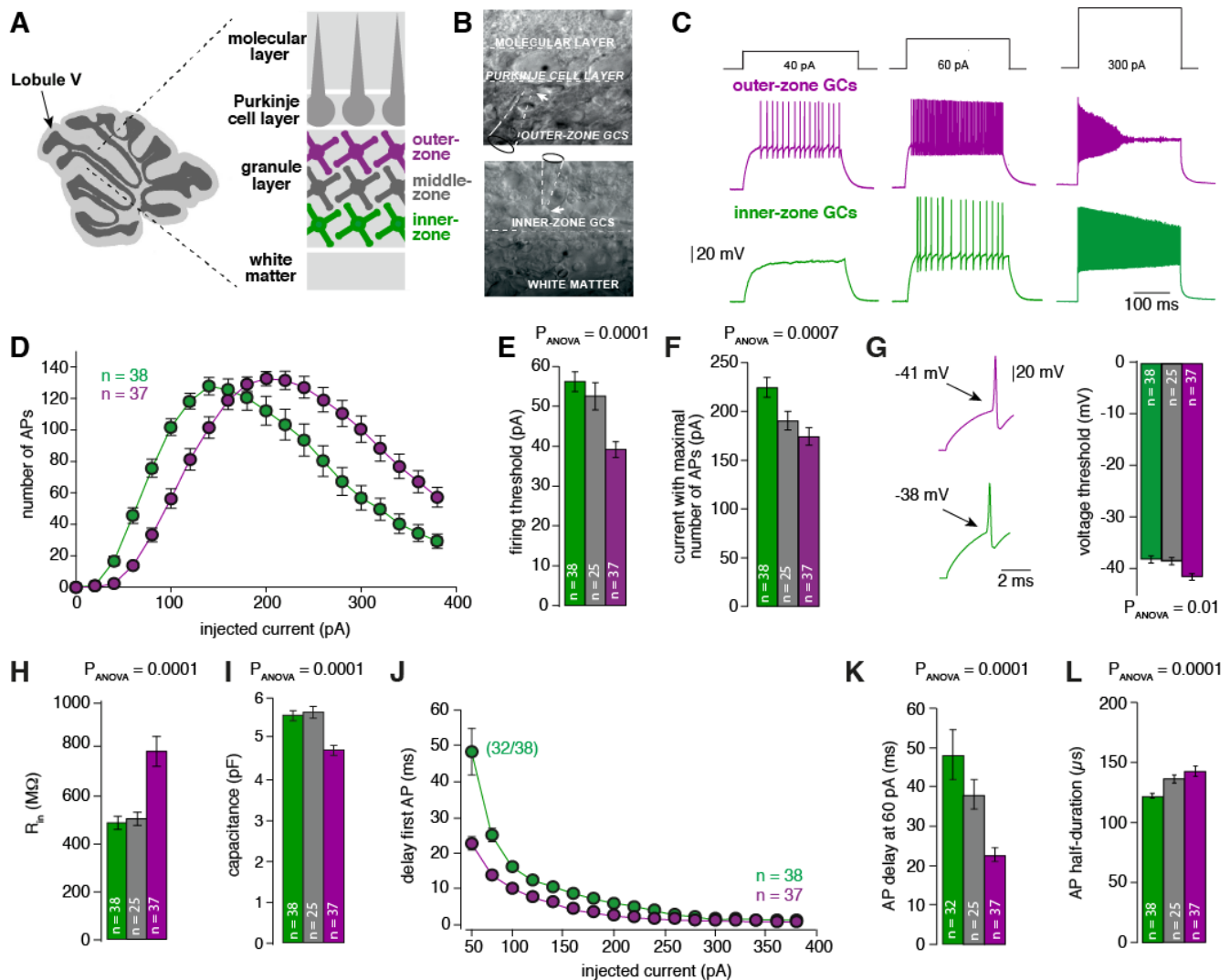
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### 870 **Statistical testing**

871 All data are expressed as mean  $\pm$  SEM. The number of analyzed cells is  
872 indicated in the figures. To test for statistically significant differences of normally

873 distributed data, we performed ANOVA or Student's t-tests and provided the P  
874 value ( $P_{ANOVA}$ , and  $P_{T-TEST}$ , respectively) above the bar-graphs. To test the  
875 differences between single groups we performed a Tukey post-hoc test and  
876 provided the corresponding P value in the figure legend ( $P_{Tukey}$ ). For not normally  
877 distributed data we performed a Mann-Whitney test.  
878

879 **Figures and figure legends:**

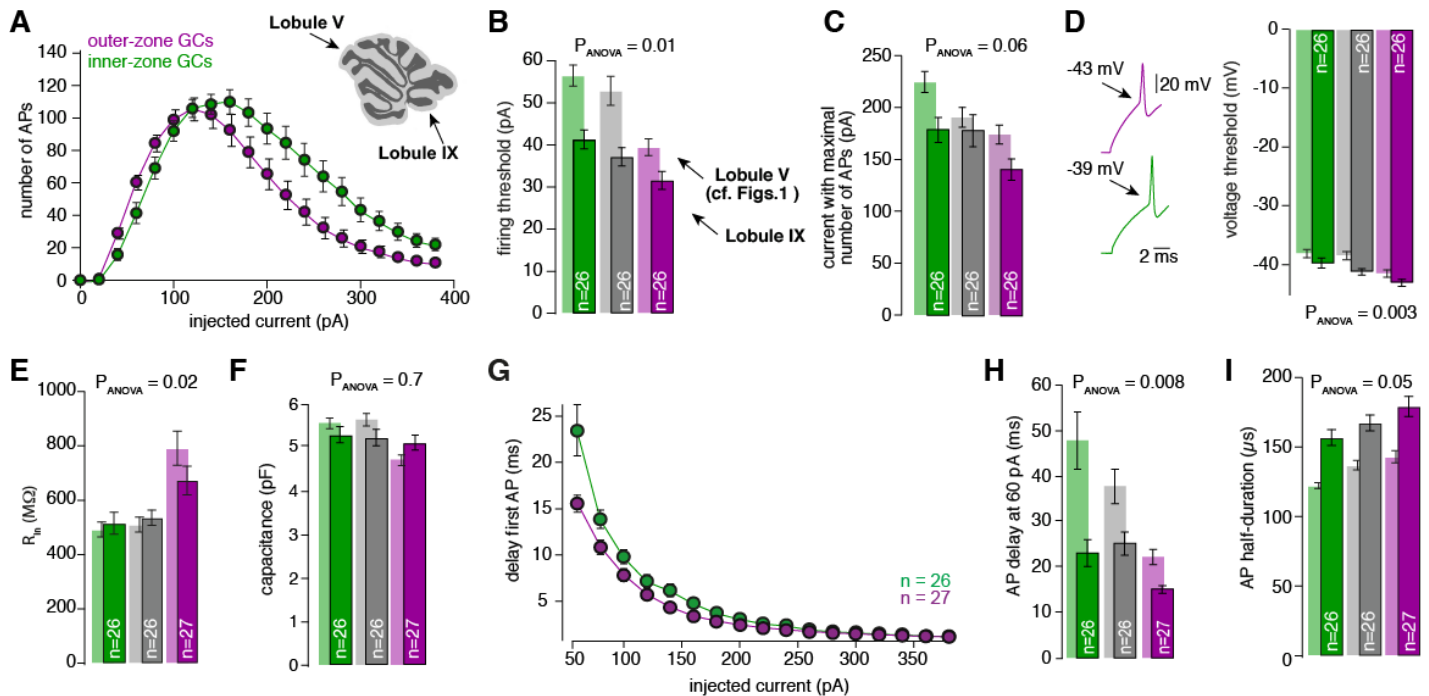


881 **Figure 1: A gradient in the biophysical properties of inner- to outer-zone**  
 882 **GCs**

- 883 A. Scheme of a parasagittal slice from the cerebellar cortex where lobule V  
 884 is indicated by an arrow. Enlargement shows a schematic  
 885 representation of the white matter, the GC, PC and molecular layer of  
 886 the cerebellar cortex. Throughout the manuscript, inner-zone GCs  
 887 (close to the white matter) are depicted in green, the middle-zone GCs  
 888 in grey, and the outer-zone GC (close to the PCs) in magenta.
- 889 B. Example differential-interference-contrast (DIC) microscopic images of  
 890 acute cerebellar slices during recordings from outer- (top) and an  
 891 inner-zone GCs (bottom). The pipette is indicated with dashed line.

- 892 C. Example current-clamp recordings from an outer-zone GC (magenta,  
893 top) and an inner-zone GC (green, bottom) after injection of increasing  
894 currents (40 pA, 60 pA and 300 pA).
- 895 D. Number of action potentials from inner- (green,  $n = 38$ ) and outer-  
896 (magenta,  $n = 37$ ) zone GCs plotted against the injected current. Note  
897 that the maximum number of action potentials is similar but outer-zone  
898 GCs achieved the maximum firing rate with a lower current injection.
- 899 E. Average current threshold for action potential firing of inner- (green),  
900 middle- (grey) and outer-zone GCs (right) ( $P_{\text{Tukey}} = 0.0001$  for inner- vs  
901 outer-zone GCs).
- 902 F. Average current needed to elicit the most number of action potentials  
903 for of inner- (green), middle- (grey) and outer-zone GCs (magenta)  
904 ( $P_{\text{Tukey}} = 0.0005$  for inner- vs outer-zone GCs).
- 905 G. Left: example action potentials from an inner- and outer-zone GC with  
906 the indicated (arrows) mean voltage-threshold for firing action  
907 potentials. Right: Comparison of the average voltage threshold for  
908 action potential firing ( $P_{\text{Tukey}} = 0.002$  for inner- vs outer-zone GCs).
- 909 H. Average input resistance of inner- (green), middle- (grey) and outer-  
910 zone GCs ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-zone GCs).
- 911 I. Average capacitance of inner-, middle- and outer-zone GCs ( $P_{\text{Tukey}} =$   
912  $0.0001$  for inner- vs outer-zone GCs).
- 913 J. Delay time of the first action potential plotted against the injected  
914 current. Note, only 32 of 38 inner-zone GCs were firing action potential  
915 at a current injection of 60 pA.
- 916 K. Delay of the first action potential of inner- (green), middle- (grey) and  
917 outer-zone GCs at a current injection of 60 pA ( $P_{\text{Tukey}} = 0.0001$  for  
918 inner- vs outer-zone GCs).
- 919 L. Average action potential half-duration of inner- (green), middle- (grey)  
920 and outer-zone GCs ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-zone GCs).  
921





923 **Figure 1—figure supplement 1: The gradient in the biophysical properties**  
 924 **of GCs and PFs is preserved throughout the cerebellar cortex.**

- 925 A. Number of action potentials (APs) from inner (green) and outer  
 926 (magenta) zone GCs plotted against the injected current. The  
 927 maximum number of action potentials is similar but with more current  
 928 injection, inner-zone GCs fire more action potentials. Inset: An image  
 929 of a cerebellar slice shows where the lobule V and lobule IX are  
 930 indicated by arrows.
- 931 B. Bar graphs represent the firing threshold of GCs from inner (dark-  
 932 green), middle (dark-grey) and outer-zone (dark-magenta). The light-  
 933 colored bar graphs in the background are the data from lobule V  
 934 shown in Figure 1. Firing threshold is higher in inner- compared to  
 935 outer-zone GCs from lobule IX, and with the same current injection,  
 936 GCs from lobule IX fire action potentials faster compared to lobule V.  
 937 The numbers of recorded GCs for lobule IX ( $n$ ) are indicated ( $P_{Tukey} =$   
 938  $0.007$  for inner- vs outer-zone GCs).
- 939 C. Average current needed to elicit the maximum number of action  
 940 potentials for of inner- (green), middle- (grey) and outer-zone GCs  
 941 (magenta) ( $P_{Tukey} = 0.002$  for inner- vs outer-zone GCs).
- 942 D. Left: example action potentials from an inner- and outer-zone GC with  
 943 the indicated (arrows) mean voltage threshold for firing action

944 potentials. Right: Voltage threshold to elicit action potentials in inner-,  
945 middle- and outer-zone GCs from lobule IX compared with data from  
946 lobule V. Voltage threshold for outer-zone GCs is lower compared to  
947 inner-zone GCs from lobule IX ( $P_{\text{Tukey}} = 0.002$  for inner- vs outer-zone  
948 GCs).

949 E. Input resistance of GCs from outer-zone of lobule IV is higher  
950 compared to inner- and middle zone GCs. But there is no difference  
951 between the input resistance of GCs from lobule V and IX ( $P_{\text{Tukey}} =$   
952  $0.02$  for inner- vs outer-zone GCs).

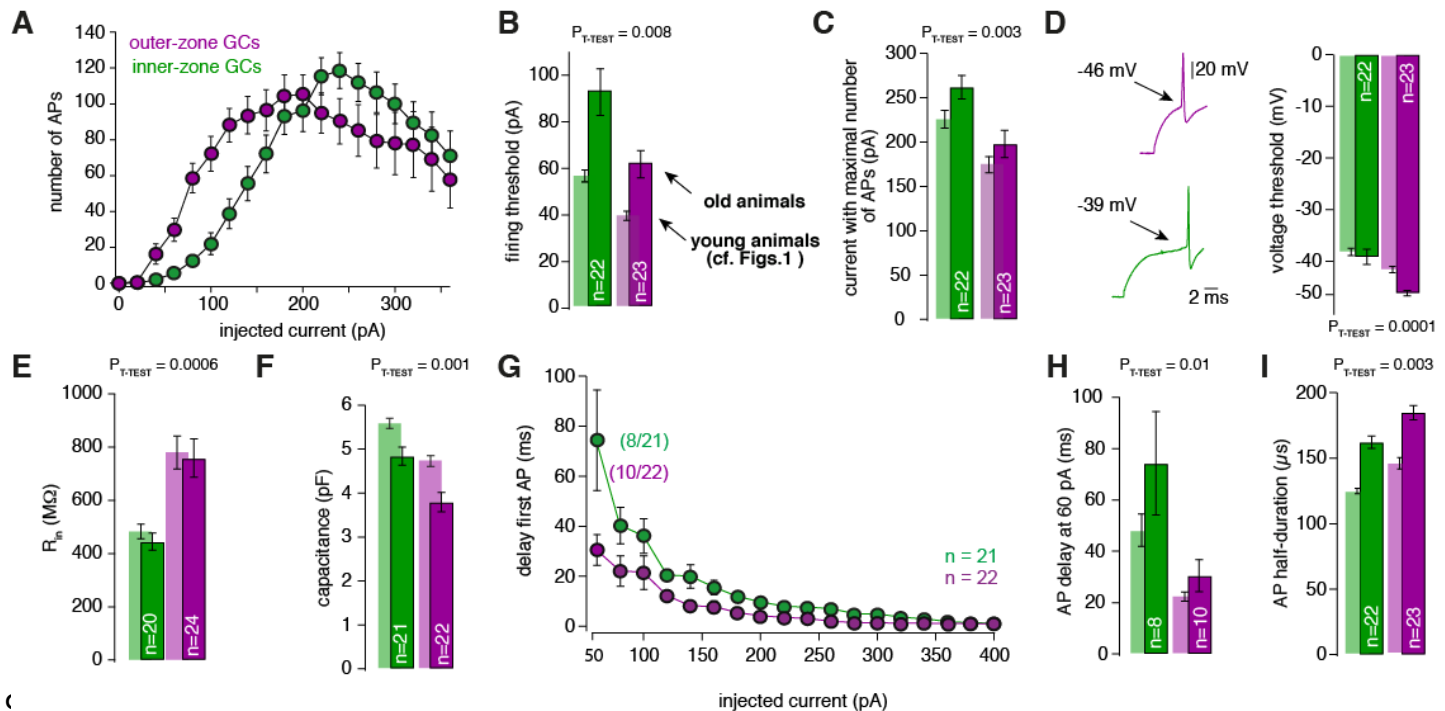
953 F. Average capacitance of inner-, middle- and outer-zone GCs. In  
954 contrary to lobule V there is no difference in the capacitance of GC  
955 from inner-, middle, or outer-zone ( $P_{\text{Tukey}} = 0.7$  for inner- vs outer-zone  
956 GCs).

957 G. Delay of the first action potential plotted against the injected current.

958 H. Delay of the first action potential after a current injection of 60 pA from  
959 inner-, middle- and outer-zone GCs from lobule IX compared to lobule  
960 V ( $P_{\text{Tukey}} = 0.05$  for inner- vs outer-zone GCs).

961 I. The action potential half-duration of inner-zone (dark-green) GCs from  
962 lobule IX is shorter compared to middle (dark-grey)- and outer-zone  
963 (dark-magenta) GCs. Compared to lobule V (faded-green, faded grey  
964 and faded magenta), the GCs from lobule IX showed a broader action  
965 potential half-width ( $P_{\text{Tukey}} = 0.09$  for inner- vs outer-zone GCs).

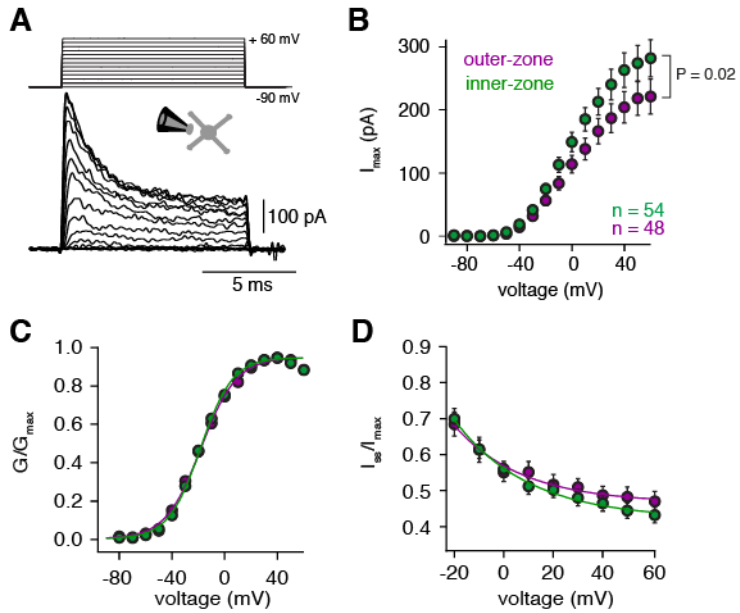
966



968 **Figure 1—figure supplement 2: The gradient in the biophysical properties**  
 969 **of GCs and PFs is also found in 3-month-old animals.**

- 970 A. Number of action potentials (APs) from inner (green) and outer  
 971 (magenta) zone GCs plotted against the injected current. The  
 972 maximum number of action potentials is similar, but with more current  
 973 injection, inner-zone GCs fire more action potentials. For all the  
 974 measurements GCs from lobule V were used.
- 975 B. Bar graphs represent the firing threshold of GCs from inner (dark-  
 976 green), and outer-zone (dark-magenta). The light-colored bar graphs  
 977 in the background are the data from lobule V in young (21-30 days-  
 978 old) animals shown in Figure 1. Firing threshold is higher in inner -  
 979 compared to outer-zone GCs from old animals.
- 980 C. Average current needed to elicit the maximum number of action  
 981 potentials for of inner- (green), and outer-zone GCs (magenta).
- 982 D. Left: example action potentials from an inner- and outer-zone GC  
 983 with the indicated (arrows) mean voltage-threshold for firing action  
 984 potentials. Right: voltage threshold to elicit action potentials in inner-  
 985 middle- and outer-zone GCs from old animals compared with data  
 986 from young animals.
- 987 E. Input resistance of GCs from outer-zone is higher compared to inner-  
 988 zone GCs. But there is no difference between the input resistance of  
 989 GCs from young and old animals.

- 990 F. Average capacitance of inner- and outer-zone GCs. In agreement with  
991 the data from the young animals, inner-zone GCs have a higher  
992 capacitance compared to outer-zone GCs.
- 993 G. Delay of the first action potential plotted against the injected current.  
994 Since the mean current threshold is higher compared to young  
995 animals only 8 out of 21 GCs from inner- and 10 out of 22 GCs from  
996 outer-zone already fired action potentials at a current injection
- 997 H. Delay of the first action potential after a current injection of 60 pA from  
998 inner-, middle- and outer-zone GCs from lobule IX compared to lobule  
999 V.
- 1000 I. The action potential half-duration of inner-zone (dark-green) GCs from  
1001 old animals is shorter compared with outer-zone (dark-magenta) GCs.  
1002

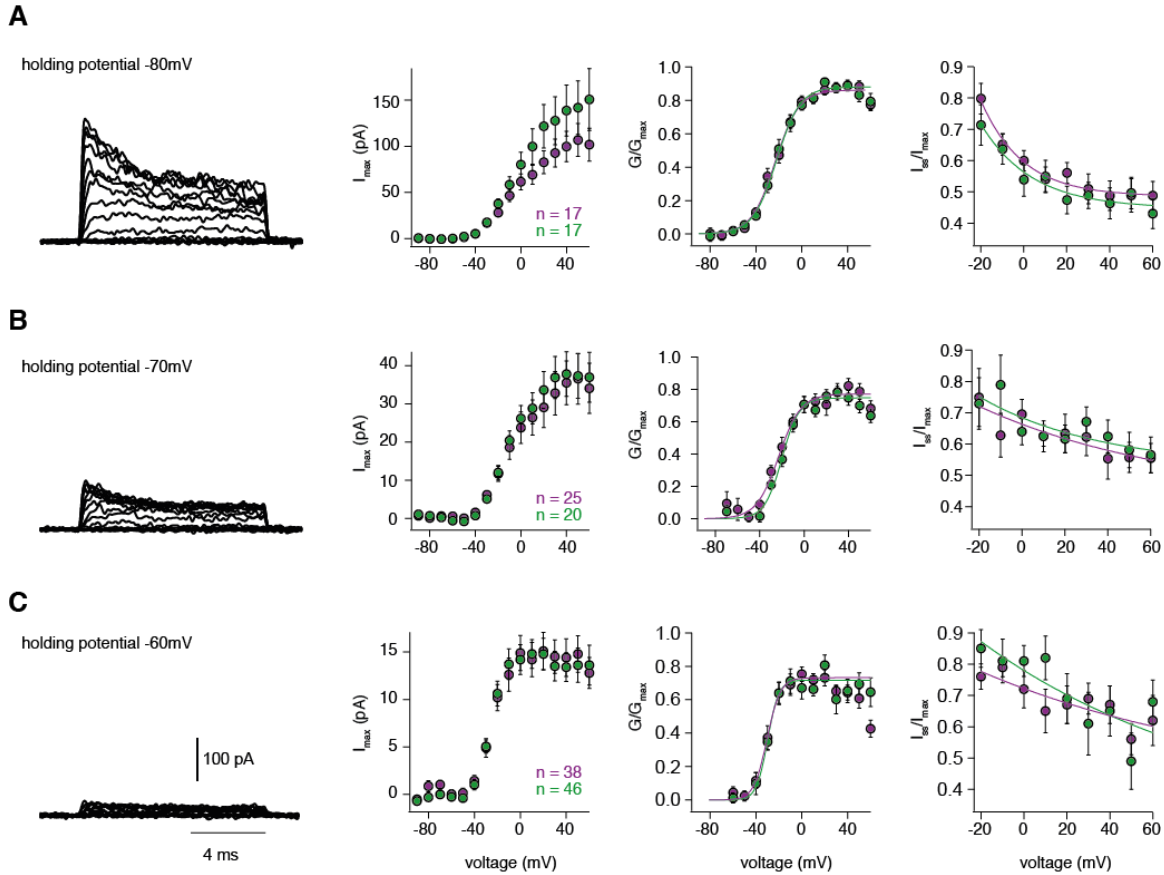


1003

1004 **Figure 2: Voltage-gated potassium currents are larger at inner-zone GCs**

- 1005 A. Example potassium currents from outside-out patches of cerebellar  
1006 GCs evoked by voltage steps from -90 to +60 mV in 10 mV increments  
1007 with a duration of 10 ms. All recordings were made in the presence of  
1008 1  $\mu$ M TTX and 150  $\mu$ M CdCl<sub>2</sub> to block voltage-gated sodium and  
1009 calcium channels, respectively.
- 1010 B. Average peak potassium current ( $I_{max}$ ) plotted versus step potential of  
1011 inner (green) and outer-zone (magenta) GCs. Significance level was  
1012 tested with a Mann-Whitney Test and the P value is indicated in the  
1013 figure. Note, that the P value shows the significance level at 60 mV.
- 1014 C. Average normalized peak potassium conductance ( $G/G_{max}$ ) versus  
1015 step potential of inner (green) and outer-zone (magenta) GCs.
- 1016 D. Average steady-state current ( $I_{ss}$ , mean current of the last 2 ms of the  
1017 10ms depolarization) normalized to the peak current ( $I_{max}$ ) versus step  
1018 potential of inner (green) and outer-zone (magenta) GCs.

1019



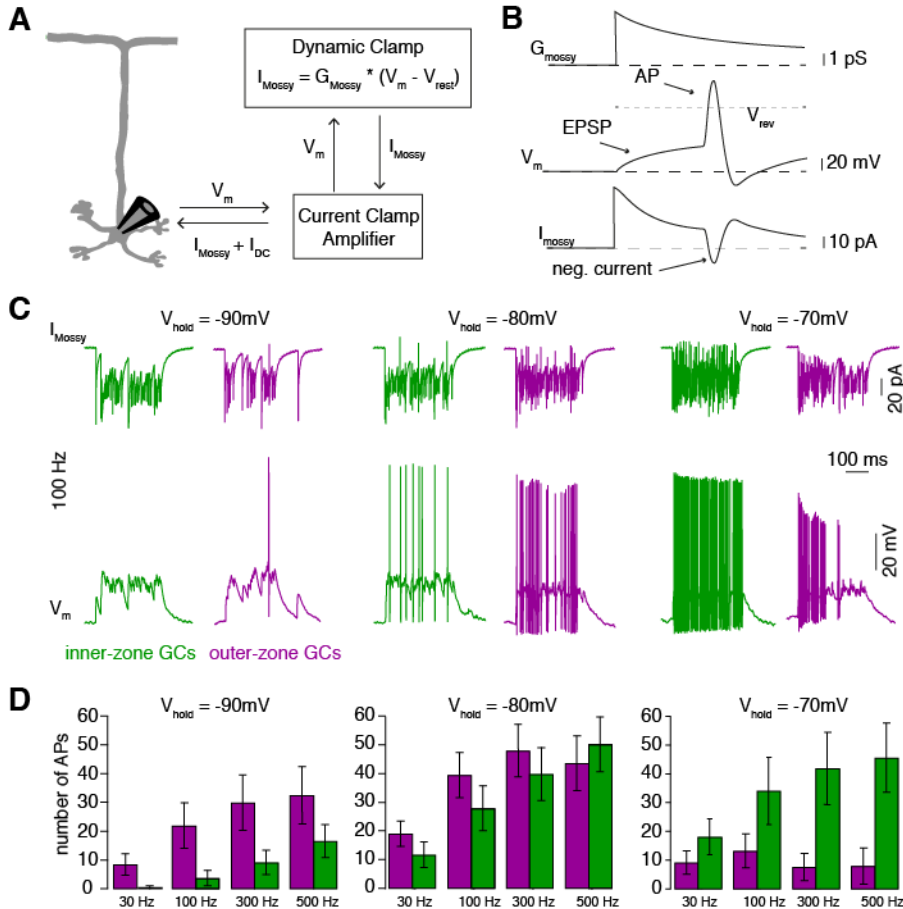
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1021 **Figure 2—figure supplement 1: Steady-state activation and inactivation are**  
1022 **similar for inner and outer GCs.**

1023

1024 A – C left: Example potassium currents from outside-out patches of cerebellar  
1025 GCs evoked by voltage steps from -90 to +60 mV in 10 mV increments with a  
1026 duration of 10 ms. The intersweep holding potential varied between -80 mV (A), -  
1027 70 mV (B) and -60 mV (C). Panels show the corresponding current-voltage  
1028 relationship (first panel) of inner-zone (green) and outer-zone (magenta) GCs,  
1029 the normalized conductance (second panel) and the normalized inactivation  
1030 behavior (third panel). The number of measured cells are indicated in the figure.

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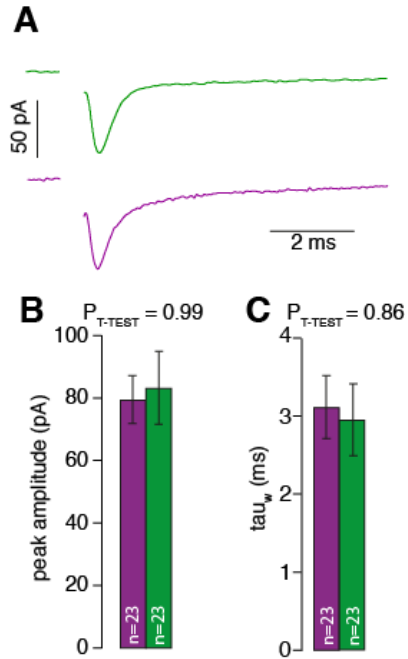
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**Figure 3: MF inputs are differentially processed by inner and outer GCs**

- Schematic representation of the Dynamic Clamp system.
- Illustration of MF conductance ( $G_{mossy}$ ), GC membrane potential ( $V_m$ ), and MF current ( $I_{mossy}$ ) for the dynamic clamp technique. Note the prediction of a negative current during the action potential as apparent in the experimental traces in panel C.
- Example dynamic clamp recordings of inner- (green) and outer-zone (magenta) GCs at different holding potentials (-90 mV left; -80 mV middle and -70 mV right) at a stimulation frequency of 100 Hz. Upper trace represents the poisson-distributed MF-like EPSCs. Lower trace shows the measured EPSPs and action potentials.
- Average number of measured action potentials (APs) after mossy-fiber like EPSC injection at different frequencies and the indicated holding potentials.



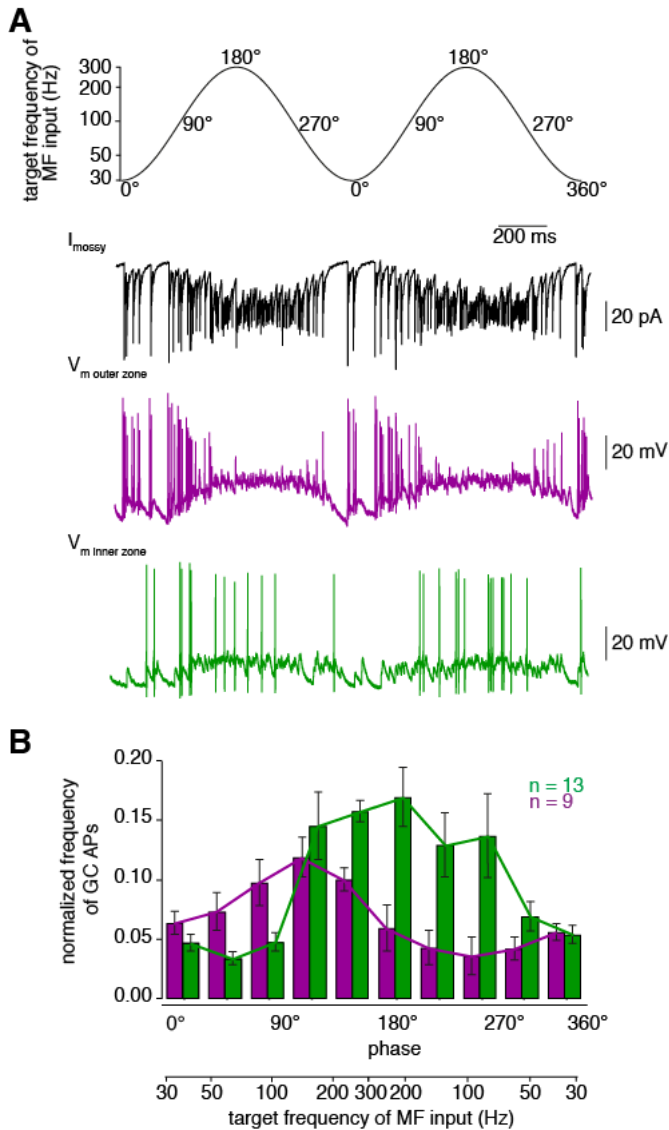
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**Figure 3—figure supplement 1: MF input is similar for inner- and outer-zone GCs.**

1053 A. Examples of single EPSCs measured from inner- (green) and outer-  
1054 zone GCs (magenta) after 1Hz stimulation of MF axons. Stimulation  
1055 artifacts were removed.  
1056 B. Average amplitude of EPSCs from inner- (green) and outer-zone GCs.  
1057 C. Weighted decay time (see material and methods) of EPSCs from  
1058 inner- (green) and outer-zone (magenta).

1059



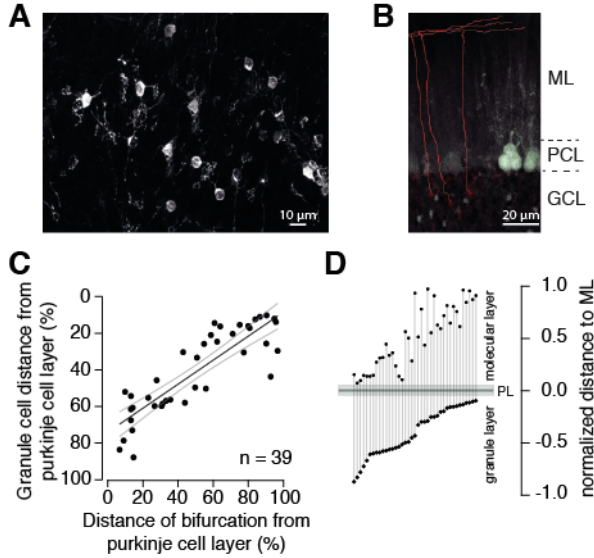


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1061 **Figure 4: Fourier-like transformation of MF input frequency**

1062 A Target frequency of the dynamic clamp MF-like sinusoidal EPSC input  
1063 current. The frequencies ranged from 30 to 300 Hz and the duration  
1064 was 1s. The degree values denote the phase angle. *Black*: example  
1065 trace of poisson-distributed MF-like input current. *Magenta and green*:  
1066 Dynamic clamp measurement of outer- and inner-zone GCs,  
1067 respectively, after stimulation with a MF-like current shown in black at a  
1068 holding potential of approximately -70 mV.

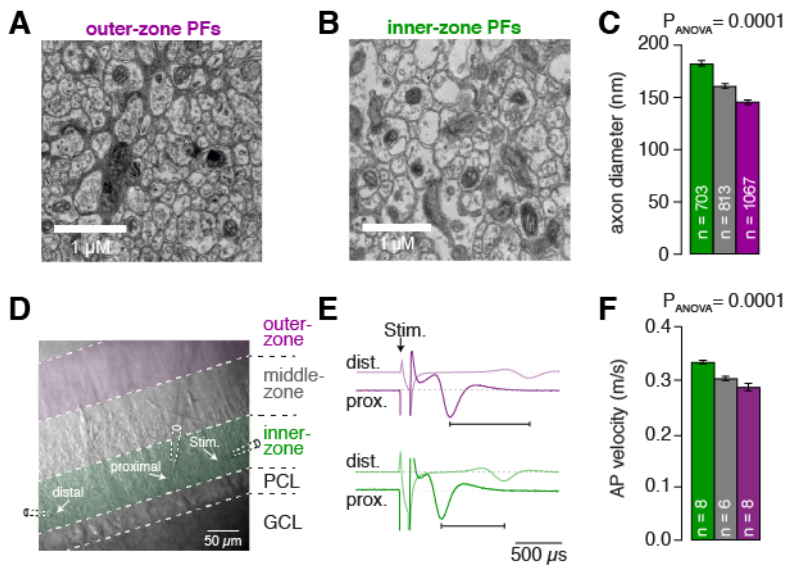
1069 B Histogram of the normalized frequency of action potentials (APs) fired  
1070 by GCs at different time intervals during 1s stimulation with poisson-  
1071 distributed MF-like currents shown in A. Each bar represents a time  
1072 window of 100 ms. Green: inner-zone GCs, magenta: outer-zone GCs.



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1075 **Figure 5: The position of PFs is correlated with the position of GC somata**

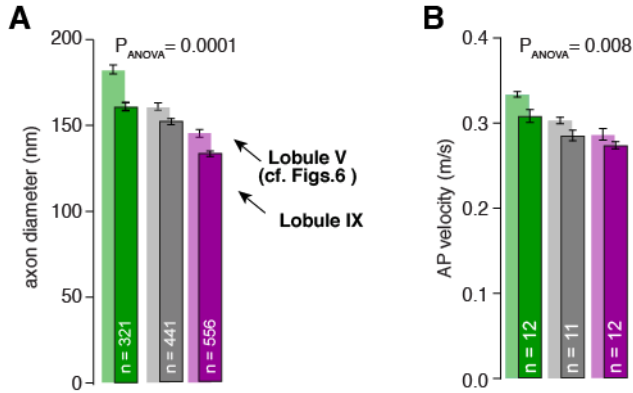
- 1076 A. Example of GCs labeled with Dil 24 h post injection. Numerous GCs  
1077 from inner-, middle-, and outer-zone were labeled.
- 1078 B. Example of traced axons from different GCs from the outer zone. The  
1079 axon was traced (red) from the cell soma to the bifurcation side in the  
1080 molecular layer. Stained cell bodies of PFs are also visible (white). *ML*:  
1081 molecular layer; *PCL*: Purkinje cell layer; *GCL*: granule cell layer.
- 1082 C. The distance between labeled GCs and the PC layer strongly  
1083 correlated with the distance between the axon bifurcation and the PC  
1084 layer (Pearson's correlation coefficient  $r = -0.862$ ;  $p < 0.001$ ). Solid black  
1085 line depicts the linear interpolation and the grey lines represent SEM of  
1086 the fit. The number of recorded GCs ( $n$ ) is indicated.
- 1087 D. Position of the GC somata from the granular layer of each traced cell  
1088 linked to the position of the bifurcation side in the molecular layer. To  
1089 avoid variability, all the distances were normalized to the  
1090 corresponding molecular layer height.  
1091



1092

1093 **Figure 6: Inner-zone PFs have larger diameter and higher action potential**  
1094 **propagation velocity.**

- 1095 A. Electron microscopic image of the outer (A) and inner zone (B) of sagittal  
1096 sections through the molecular layer.
- 1097 C. Summary of axon diameters in the inner (green), middle (grey), and outer  
1098 zone (magenta) of the molecular layer ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-  
1099 zone GCs).
- 1100 D. DIC image of the molecular layer superimposed with a schematic  
1101 illustration of the experimental setup to measure compound action  
1102 potentials from PFs. Compound action potentials were evoked by a  
1103 stimulus electrode (left) and recorded by a proximal and distal recording  
1104 electrode (middle, right).
- 1105 E. Example traces used to determine the conduction velocity between inner-,  
1106 middle-, and outer-zone PFs. The time difference between the compound  
1107 action potential arriving at the proximal electrode (solid traces) and the  
1108 distal electrode (faint traces) was used to determine the velocity. The time  
1109 was shorter for inner-zone PFs (green) compared with outer-zone PFs  
1110 (magenta).
- 1111 F. Summary of action potential (AP) velocity in inner-, middle- and outer-  
1112 zones ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-zone GCs).
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**Figure 6—figure supplement 1: Differences in axon diameter and action potential velocity are also found in lobule IX.**

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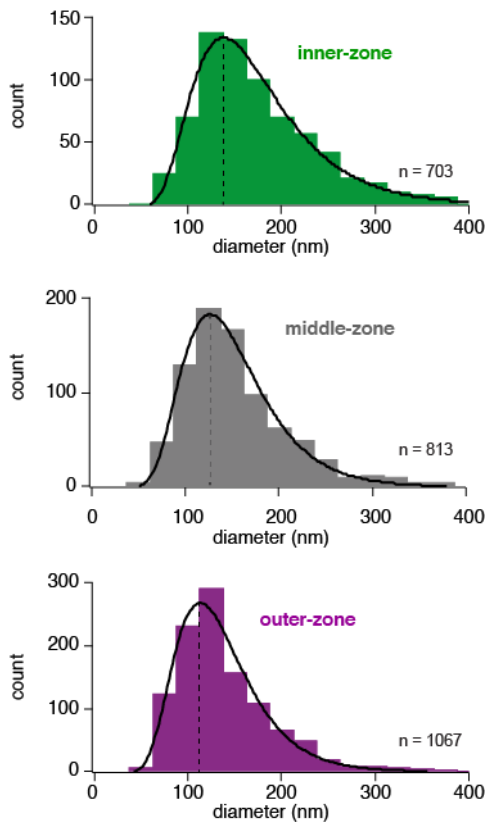
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- A. Summary of axon diameters in the inner (green), middle (grey), and outer zone (magenta) of the molecular layer ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-zone GCs). The light-colored bar graphs in the background are the data from lobule V shown in Figure 6.
- B. Summary of action potential (AP) velocity in inner-, middle- and outer-zones of PF from molecular layer of lobule IX ( $P_{\text{Tukey}} = 0.0001$  for inner- vs outer-zone GCs).

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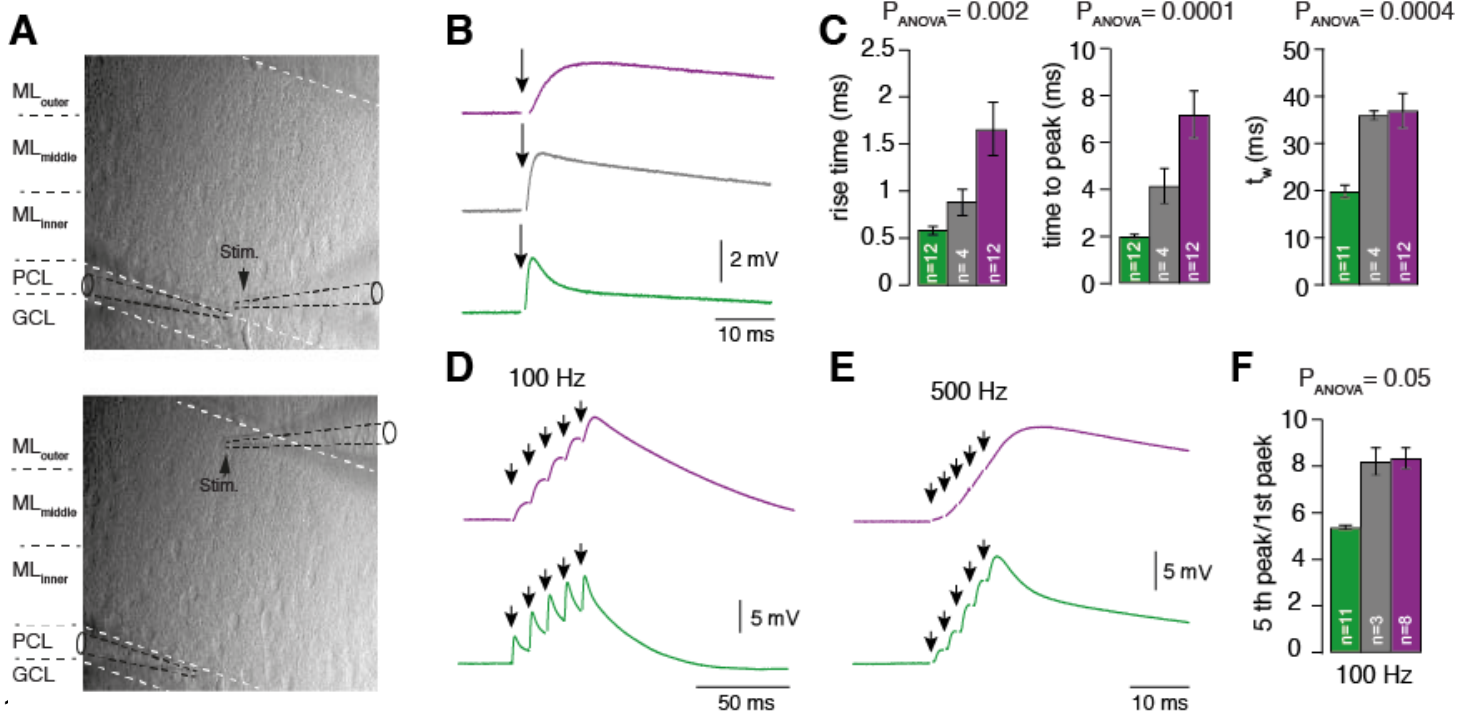
1130 **Figure 6—figure supplement 2: Histogram of the axon diameters.**

1131 Histograms of the diameter of inner-, middle- and outer-zone axons in the  
1132 molecular layer, indicating that the change in axon diameter is not due to an  
1133 increased fraction of larger diameter axons from non-GC cells (e.g. Lugaro cells).  
1134 Instead the entire distribution of the axon diameters is shifted between zones.

1135 Data were fit with a skewed Gaussian function:  $a e^{\left[-\log(2) \left( \left( \log \left( 1 + 2b \frac{d-d_0}{ds} \right) / b \right)^2 \right) \right]}$ ,  
1136 where  $a$  is the amplitude,  $d$  the diameter, and  $d_0$  the diameter at the peak.  $ds$   
1137 and  $b$  represent parameters related to the width and the skewness, respectively.  
1138 The peak is indicated by a vertical line.

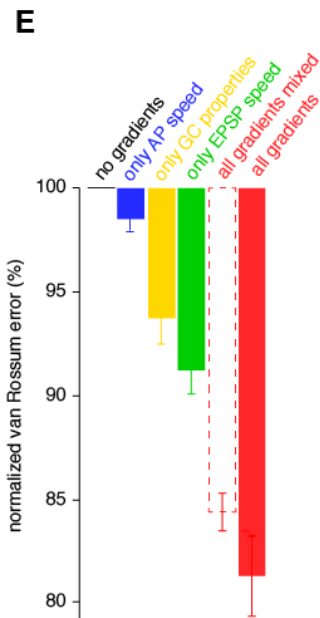
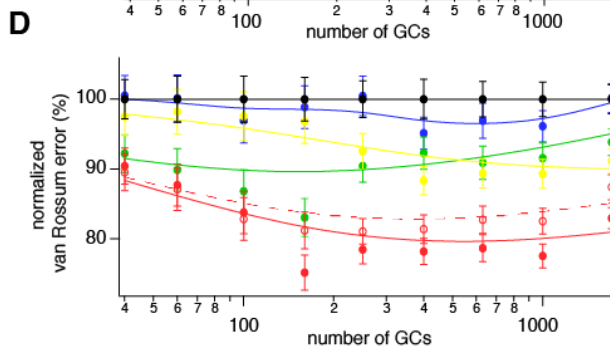
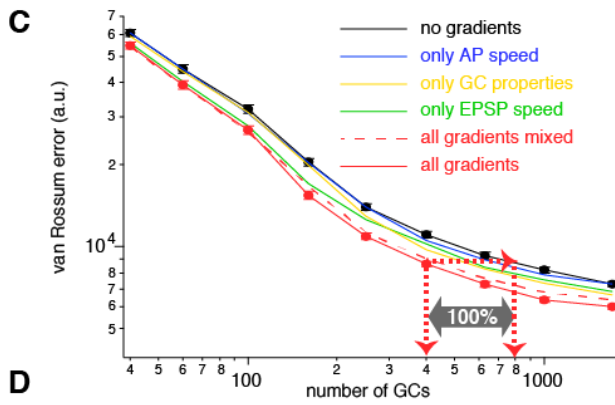
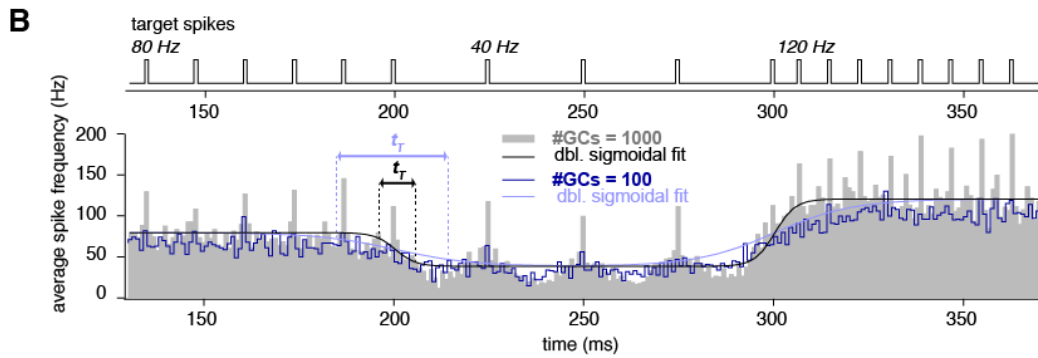
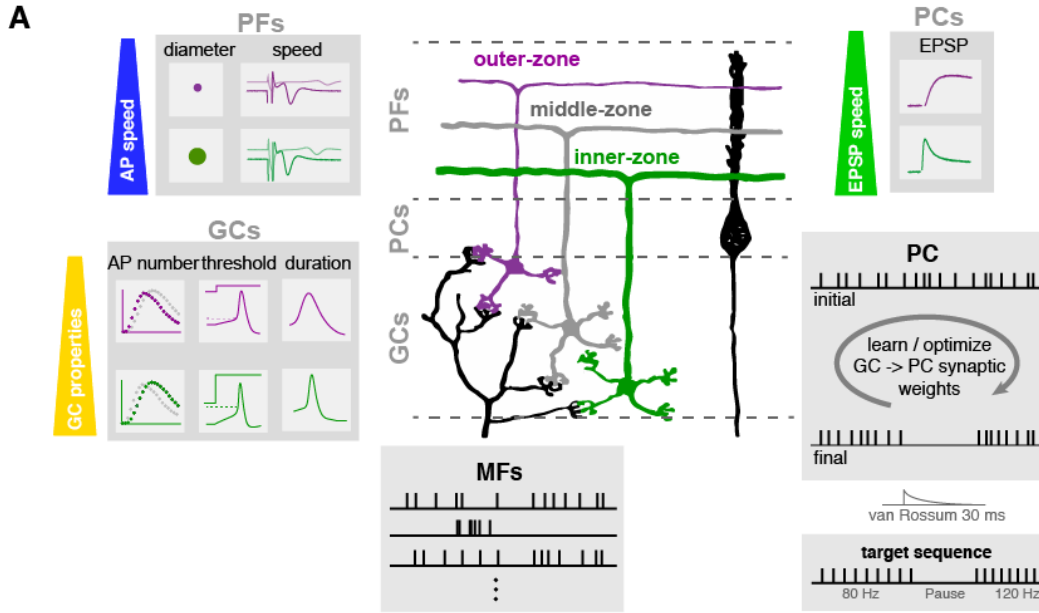
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1142 **Figure 7: PCs process inner-, middle-, and outer-zone PF inputs**  
 1143 **differentially**

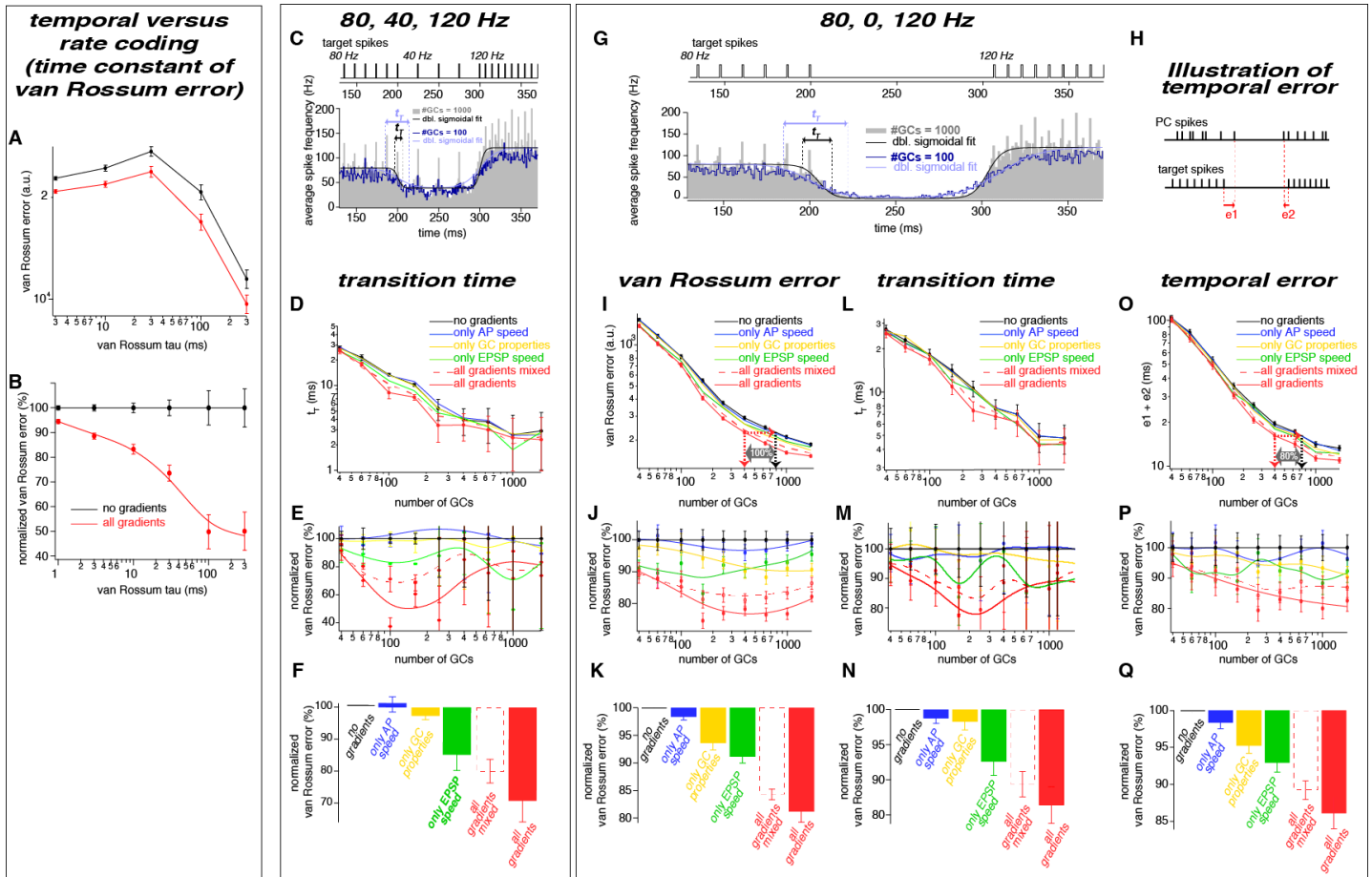
- 1144 A. DIC image of the molecular layer superimposed with a schematic  
 1145 illustration of PC recordings while stimulating inner (top) and  
 1146 outer-zone PFs (bottom). Shown are the GC layer (GL), PC layer (PC) and  
 1147 molecular layer (ML).
- 1148 B. EPSPs measured at the PC soma after stimulation (1 Hz) of inner-  
 1149 (green), middle- (grey), and outer-zone PFs (magenta).
- 1150 C. Average 20% to 80% rise time, time to peak and weighted time-  
 1151 constant of PC EPSPs after stimulation of inner- (green;  $n = 12$ ),  
 1152 middle- (grey;  $n = 4$ ) and outer-zone PFs (magenta;  $n = 12$ ) as shown  
 1153 in B ( $P_{Tukey} = 0.001$ ;  $P_{Tukey} = 0.0001$ ;  $P_{Tukey} = 0.0004$  for inner- vs outer-  
 1154 zone GCs, respectively).
- 1155 D-E. Example traces of EPSPs from a PC after five impulses to inner-  
 1156 (green) and outer-zone PFs (magenta) at 100 Hz (D) and 500 Hz (E).
- 1157 F. Average paired-pulse ratio measured in PCs after five 100-Hz stimuli  
 1158 at inner- (green;  $n = 11$ ), middle- (grey,  $n = 3$ ) and outer- zone PFs  
 1159 (magenta,  $n = 8$ ) ( $P_{Tukey} = 0.054$  for inner- vs outer-zone GCs).  
 1160



1162 **Figure 8: The observed neuronal gradients increase storing capacity and**  
1163 **improve temporal precision of PC spiking**

- 1164 A. Schematic illustration of the network model of the cerebellar cortex as  
1165 explained in the main text.
- 1166 B. Average spiking histogram for models consisting of 100 and 1000  
1167 GCs, superimposed with double sigmoidal fits constrained to 80, 40  
1168 and 120 Hz. Above the target spiking sequence is indicated.  $t_7$   
1169 indicates the transition time of the sigmoidal fit for the respective  
1170 number of GCs.
- 1171 C. Double logarithmic plot of the average minimal van Rossum error  
1172 plotted against the number of GCs for a model with no gradients  
1173 (black), a model with only gradually varied GC parameters (yellow), PF  
1174 propagation speed (blue), EPSP kinetics (green), and all gradients  
1175 (red). Furthermore, all parameters were gradually varied but the  
1176 connectivity between GC, PF and EPSPs was random (all gradients  
1177 mixed; dashed red). Red dashed lines with arrows indicate the number  
1178 of GCs needed to obtain the same van Rossum error with all gradients  
1179 compared to no gradients. With no gradients 100% more GCs are  
1180 needed to obtain the same van Rossum error.
- 1181 D. Average van Rossum errors as shown in panel C but normalized to the  
1182 value of the model without gradients, superimposed with a smoothing  
1183 spline interpolation.
- 1184 E. Error bar indicate average of the relative differences shown in panel D.
- 1185

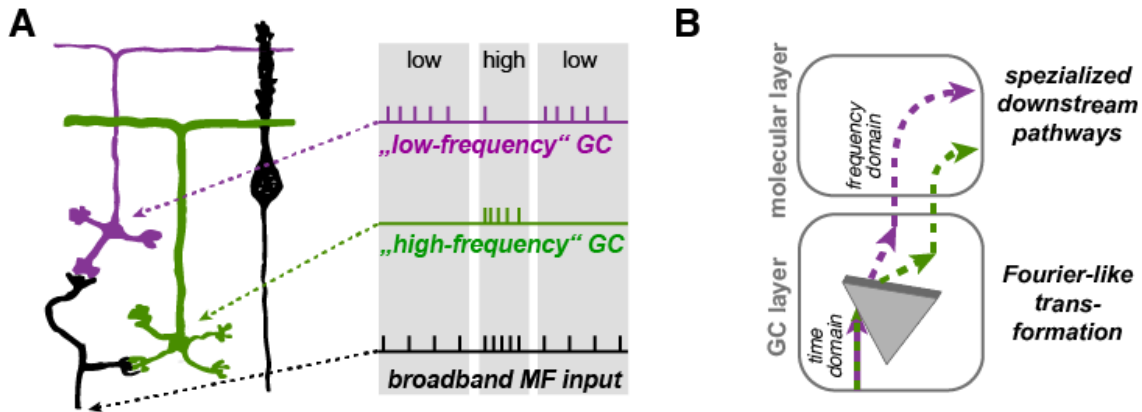




1187 **Figure 8—figure supplement 1: The observed neuronal gradients reduce**  
 1188 **the temporal error and improve rate coding of PC spikes.**

- 1189 A. Double logarithmic plot of the van Rossum error of model with 300  
 1190 GCs without (black) and with all gradients (red) plotted versus the time  
 1191 constant of the van Rossum kernel ranging from 2 to 300 ms.  
 1192 B. Data as in panel A normalized to the model without gradients.  
 1193 C. Average spiking histogram for models consisting of 100 and 1000  
 1194 GCs, superimposed with double sigmoidal fits constrained to 80, 40  
 1195 and 120 Hz. Above the target spiking sequence is indicated.  
 1196 D. Double logarithmic plot of the transition time ( $t_T$ ) of the double  
 1197 exponential fits as illustrated in panel C. Error bars represent 95%  
 1198 confidence intervals.  
 1199 E. Transition time ( $t_T$ ) as shown in panel D but normalized to the value of  
 1200 the model without gradients, superimposed with a smoothing spline  
 1201 interpolation.  
 1202 F. Average of the relative differences shown in panel E.  
 1203 G. Average spiking histogram for models consisting of 100 and 1000  
 1204 GCs, superimposed with double sigmoidal fits constrained to 80, 0 and

1205                    120 Hz. Above the target spiking sequence with 80, 0, and 120 Hz is  
1206                    indicated.  
1207                    H.    Illustration of the temporal error ( $e_1$  and  $e_2$ ) of the spikes defining the  
1208                    beginning and the end of the pause.  
1209                    I-K, L-N, and O-Q. Same plots as D-F but for the 80, 0, 120 Hz target  
1210                    sequence and the van Rossum error (I-K), the transition time ( $t_T$ ) (L-N) and  
1211                    the temporal error (O-Q).  
1212  
1213



1214

1215 **Figure 9: Illustration of the concept of Fourier-like transformation in the**  
1216 **cerebellar cortex**

- 1217 A. Illustration of a broadband MF inputs conveying a sequence of low,  
1218 high, and low firing frequency. Inner-zone GCs will preferentially fire  
1219 during high-frequency inputs ('high-frequency' GC) and outer-zone  
1220 GCs during low-frequency inputs ('low-frequency' GC).
- 1221 B. Schematic illustration of the signal flow through the cerebellar cortex.  
1222 The Fourier-like transformation in the GC layer is illustrated as an  
1223 optical prism separating the spectral components on the MF input.  
1224 Thereby the MF signal in the time domain is transformed to the  
1225 frequency domain and sent via specialized signaling pathways in the  
1226 molecular layer to the PC.

1227

1228 **References**

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