Figures and figure supplements

Cerebellar modules operate at different frequencies

Haibo Zhou, et al.
Figure 1. Simple spike firing activity differs between Purkinje cell populations. (A) Extracellular recordings were made from PCs in the cerebellar cortex of awake mice, using double barrel glass electrodes (right). Dye injections were placed to histologically identify the recording location. (B) Photomicrographs of coronal sections with examples of zebrin-negative (Z−, left) and zebrin-positive (Z+, right) identified Purkinje cells in lobule II and lobule IX, respectively. Cells are marked by dye injections (blue, indicated by arrows), zebrin is stained brown, dotted lines demark zebrin borders. Note that Z+ stripes in lobules I–III are very narrow. (C and D) Example trace of Z− and Z+ Purkinje cell recordings identified by its hallmark feature, the occurrence of complex spikes (asterisk) and simple spikes. (E) Recordings were confirmed to be from a single neuron by the consistent pause in simple spike firing following each complex spike, in the overlay. (F) Overlay of simple spikes. (G) Distribution of recorded Z− and Z+ cells throughout the unfolded cerebellar cortex based on zebrin II compartments. (H) Simple spike firing frequency is significantly lower in identified Z+ PCs compared to Z− PCs (Z−: n = 47 cells, 26 mice; Z+: n = 57 cells, 34 mice; t = 9.942, p<0.001). (I) In line with the lower simple spike firing frequency, the climbing fiber pause was longer in identified Z+ Purkinje cells (CF pause; t = −7.482, p<0.001). (J) Simple spike regularity is not different between Z+ and Z− PCs (CV2: t = 1.147, p=0.234). Error bars represent SD, *p<0.05, **p<0.001. Schematic drawing in A was adapted from Sugihara and Quy (2007) with permission. Scale bars in A and B indicate 100 and 200 µm, respectively.

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Figure 1—figure supplement 1. Experimental approach and histological verification.
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Figure 1—figure supplement 2. Stability of key parameters over the recording time.
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Figure 2: Simple spike firing frequency correlates with the zebrin identity of Purkinje cells. To determine if the differences in simple spike activity are related to the location of the Purkinje cells, or to their zebrin identity, we compared PC activity of Z+ against Z− Purkinje cells in various smaller areas of the cerebellar cortex. (A) To more directly test the link with zebrin, we used EAAT4-eGFP mice that express eGFP in a pattern similar to that of zebrin. Two-photon images show an EAAT4+/Z+ band (green) in lobule V of an EAAT4-eGFP mouse: left, electrode (blue) positioned in the adjacent negative band; right, electrode (blue) in the positive band. (B) The activity of 17 zebrin-identity determined PCs (Z+, n = 8; Z−, n = 9, 5 mice) from lobule V, VI, and Crus I was recorded. (C) The difference in simple spike firing frequency was pertained in this subset of Purkinje cell recordings (Z+: 36.0 ± 15.5 Hz; Z−: 75.8 ± 19.5 Hz; t = 4.618, p<0.001), indicating that this difference is linked to zebrin identity, rather than lobular location. In contrast to data obtained with immunostaining for zebrin, the regularity of simple spikes also differs in this subpopulation (t = −2.715, p<0.016). (D) Cerebellar Purkinje cells can be subdivided based on the input they receive into four transverse zones: the anterior (red), central (orange), posterior (yellow), and nodular (green) zone. (E) The difference in simple spike firing frequency between Z+ and Z− Purkinje cells is consistently present throughout all transverse zones. In each of the four transverse zones, the simple spike rate was significantly lower in Z+ compared to Z− Purkinje cells (all p<0.05, One-tailed Student’s t test). Note that simple spike frequency within different Z+ subgroups was also variable, in that the frequency in the anterior zone was lower than that in the nodular zone (p=0.018, One-way ANOVA, followed by Bonferroni’s posthoc test). Error bars represent SD, *p<0.05, **p<0.001.

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Figure 2—figure supplement 1. Overview with color-coded simple spike frequency for all identified Z+ and Z− Purkinje cells.

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Figure 3. Zebrin staining intensity and simple spike frequency are inversely correlated. To test the correlation to zebrin identity of modules throughout the cerebellar cortex, Purkinje cell activity was recorded from all parts of the cerebellar cortex, each followed by dye injection to identify the lobule. (A1–2) The average zebrin staining intensities of Purkinje cell somata in vermis and hemispheres were obtained from the sagittal sections of three mice. Note that high intensity values equal weak staining, and vice versa. (B1–2 and C1–2) The average simple spike firing frequency (vermis: \(n = 192\) cells, 70 mice, \(r = 0.893, p=0.007\); hemisphere: \(n = 53\) cells, 30 mice, \(r = 1.000, p<0.001\)) and CF pause (vermis: \(r = -0.929, p=0.003\); hemisphere: \(r = -1.000, p<0.001\)) show significant correlation with zebrin intensity over different parts of vermis and hemisphere. (D1–2) The CV2 of SSs could not be consistently related with zebrin intensity (vermis: \(r = 0.929, p=0.003\); hemisphere: \(r = -0.300, p=0.624\)). Error bars represent SD. HIV&V-Sim, hemispheral part of lobule IV&V and simple lobule; CrI-II, Crus I and II; PM, paramedian lobule; Cop-PF, copula of the pyramis and paraflocculus; Flocc, flocculus. DOI: 10.7554/eLife.02536.008
Figure 3—figure supplement 1. Statistical analysis of PC spiking characteristics per lobule.
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Figure 4. Complex spike characteristics depend on zebrin identity. (A) Similar to simple spike frequency, complex spike frequency differs between immunostaining identified Z+ and Z− PCs (data from zebrin-identified PCs shown in Figure 1; t = 3.926, p<0.001). (B) This difference is confirmed in the sample of Z+ and Z− PCs obtained by two-photon imaging in EAAT4-eGFP mice, in that Z+ Purkinje cells have a lower complex spike firing frequency here too (t = 2.692, p=0.017). (C) Moreover, complex spikes frequency shows significant correlation with zebrin intensity in vermis and hemisphere (vermis: r = 0.929, p=0.003; hemisphere: r = 1.000, p<0.001). Even though the regularity of CSs differs between immunostaining identified Z− and Z+ PCs (A, bottom), this was not reproduced in the other two experimental data sets (B–C, bottom). (D) Typical Z− and Z+ CS shapes (−0.5 to +3 ms) showing the characteristics analyzed: half-width and spike area (left). Z+ PCs have a longer half-width and bigger spike area than Z− cells (right). (E) Raster plots of simple spike activity around complex spikes (event, −100 ms till +300 ms) were converted in peri complex-spike time histograms. Based on these histograms, we could distinguish four different types of simple spike response types among the Purkinje cells recorded in all areas: normal, facilitation, suppression and oscillation. (F) The percentage of different types in Z− and Z+ PCs (values indicate percentage). The facilitation type occurs predominantly in Z− PCs, whereas the suppression and oscillation type are restricted to the Z+ PCs. (G) Attempts to find other parameters correlating in all recorded cells (n = 243 cells) with the response type were largely unsuccessful. The exception is the oscillation type, which has a signature combination of simple spike frequency and CV (11 out of 13, SS freq. range 35–60 Hz and CV <0.32). Two-photon imaging data are only included in panel B; panels D–F are based on immunostaining identified Z+ and Z− PCs only and panels C and G on all recorded PCs. Error bars represent SD, *p<0.05, **p<0.001.

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Zebrin-related differences are present in the intrinsic activity of Purkinje cells. To test if intrinsic or input-related differences underlie the difference in simple spike frequency, we recorded PC activity in conditions of limited or no synaptic input. (A) PC activity was recorded in vitro (n = 107 cells, 15 mice) under complete block of synaptic inputs. (B) Spiking frequency in vitro (red) was lower than that in vivo (black) over the range of lobules, but the shape of the curve was similar (r = 0.916, p=0.010, Pearson’s correlation). (C1–3) To verify the correlation with zebrin, we recorded activity of EAAT4/zebrin-positive and negative PCs in slices of EAAT4-eGFP mice. Both in lobules II–V (Z+: n = 7 cells, Z−: n = 4; 3 mice; t = 2.910, p=0.017) and lobules VIII–IX (Z+: n = 6, Z−: n = 5; 2 mice; t = 2.352, p=0.043) the difference in simple spike firing frequency was present, further confirming the link with zebrin. (D) Next, extracellular recordings were made in vivo in a6-Cacna1a and PC-Δγ2 mutant mice that have minimized excitatory and no synaptic inhibitory inputs to their PCs, respectively. (E) PC activity in Z+ lobule X of both mutants was lower than that in the predominantly Z− lobules I–III (wild types, lobules I–III: n = 43 cells, 18 mice, lobule X: n = 32 cells, 25 mice, t = 6.808, p<0.001; a6-Cacna1a, I–III: n = 16 cells, 2 mice; X: n = 11 cells, 2 mice, t = 3.979, p<0.001; PC-Δγ2, I–III: n = 11 cells, 3 mice; X: n = 17 cells, 3 mice; t = 4.876, p<0.001). Inset compares the absolute differences in firing frequency between lobules I–III and X. (F) CV2 values of Z− and Z+ SS activity from in vitro recordings (lobules I–III and X: both p<0.001) and in vivo recordings of both a6-Cacna1a mutants (lobule I–III: t = 5.613, p<0.001; lobule X: t = 2.062, p=0.046) and PC-Δγ2 mutants (lobules I–III and X: both p<0.005) were significantly lower than the wild type recordings. Abbreviations: cf, climbing fiber; GC, granule cell; IO, inferior olive; MLI, molecular layer interneuron; PC, Purkinje cell; pf, parallel fiber. Error bars represent SD, *p<0.05, **p<0.001.

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Figure 5—figure supplement 1. Purkinje cell intrinsic excitability is higher in lobule III than in X. DOI: 10.7554/eLife.02536.012
Figure 6. Blocking TRPC3 attenuates the simple spike frequency difference. In search for the underlying mechanism, we tested the contribution of TRPC3, which can be indirectly linked to zebrin-like expression.

(A and B) The presence of TRPC3 blocker genistein (10 µM) or the more selective Pyr3 (100 µM) reduced Purkinje cell firing frequency in lobule III (vehicle: n = 47 cells, 6 mice; genistein: n = 25 cells, 7 mice; Pyr3: n = 33 cells, 7 mice; both p<0.001 vs vehicle, One-Way ANOVA followed by Tukey’s post-hoc test), but not in lobule X (vehicle: n = 48 cells, 6 mice; genistein: n = 44 cells, 7 mice; Pyr3, n = 24 cells, 7 mice; p=0.271 and p=1.000 vs vehicle, respectively, One-Way ANOVA followed by Tukey’s post-hoc test), virtually eliminating the difference between averages for lobule III–X (inset). To more directly quantify the effect of blocking TRPC3, we washed-in Pyr3 during the recording of Purkinje cells in lobule III and X. (C–E) Pyr3 wash-in significantly decreased the simple spike firing frequency in lobule III (n = 7 cells, 7 mice; t = 5.412, p=0.002, paired Student’s t test), and this decrease was larger in lobule III than in lobule X (t = 4.069, p=0.002). (F–G) In line with the in vitro data, in vivo blocking of TRPC3 by application of genistein (240 mg/kg, i.p.) or Pyr3 (200 µg, i.c.v.) decreased simple spike firing in lobule I–III (vehicle: n = 27 cells, 3 mice; genistein: n = 37 cells, 3 mice and Pyr3: n = 30 cells, 2 mice; both p<0.001 vs vehicle, one-way ANOVA followed by Tukey’s post-hoc test), but had no effect in lobule X (vehicle: n = 32 cells, 3 mice; genistein: n = 23 cells, 3 mice).
Figure 6. Continued

cells, 4 mice and Pyr3: n = 31 cells, 4 mice; p=0.546
and p=0.887 vs vehicle, respectively one-way ANOVA
followed by Tukey’s post-hoc test, resulting in a
pronounced reduction of the difference (inset). Error
bars represent s.d., *p<0.05, **p<0.001.
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Figure 6—figure supplement 1. Aldolase C enzymatic
reaction products GAP and DHAP increase the activity
in lobules III and X.
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Figure 6—figure supplement 2. Effects of blocking
EAAT4 on Purkinje cell activity in lobule III and X in
vitro.
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Figure 6—figure supplement 3  Effects of TRPC3 blockers on other PC activity parameters. DOI: 10.7554/eLife.02536.016