HCN1 channels in cerebellar Purkinje cell promote late stages of learning and constrain synaptic inhibition

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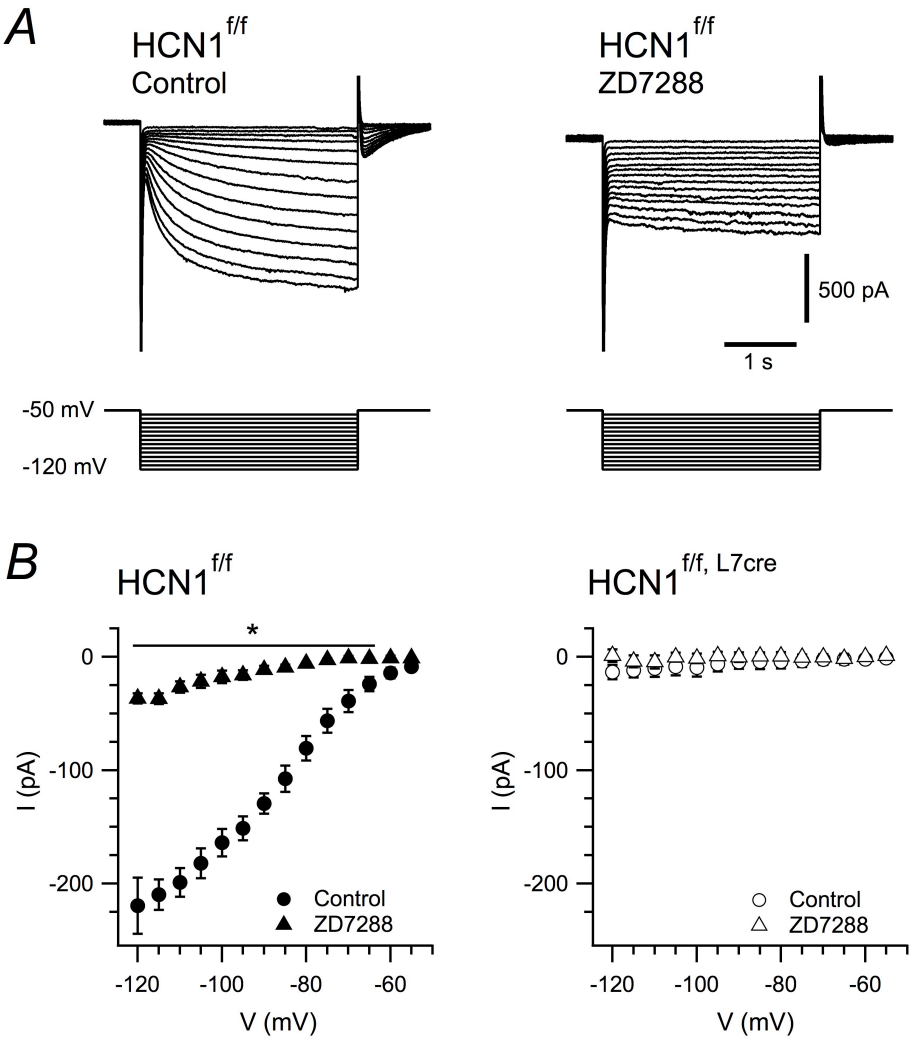
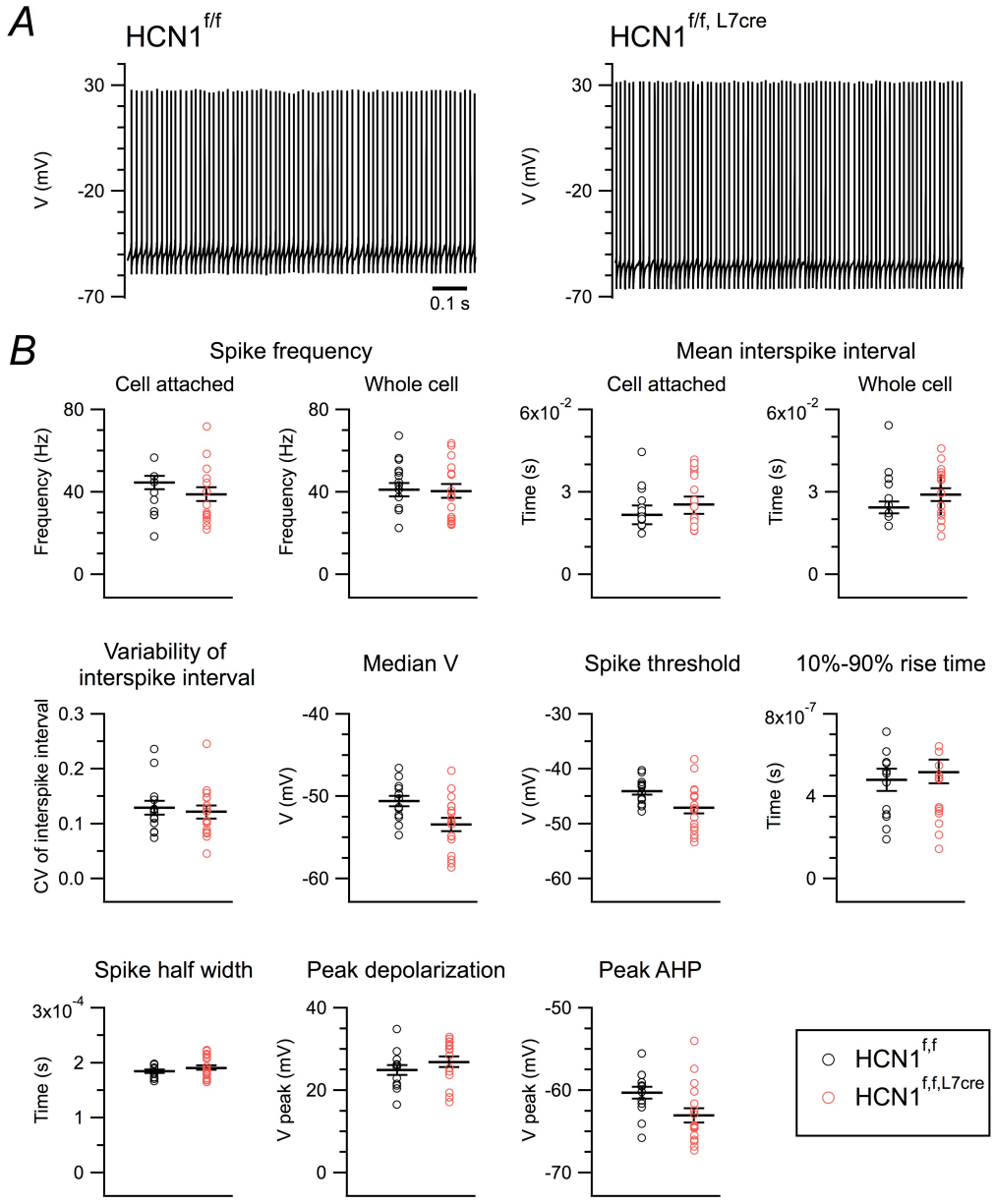
Supplemental Figures 1-7 and Supplemental Data



Supplemental Figure 1. Deletion of HCN1 from Purkinje cells abolishes I_h.

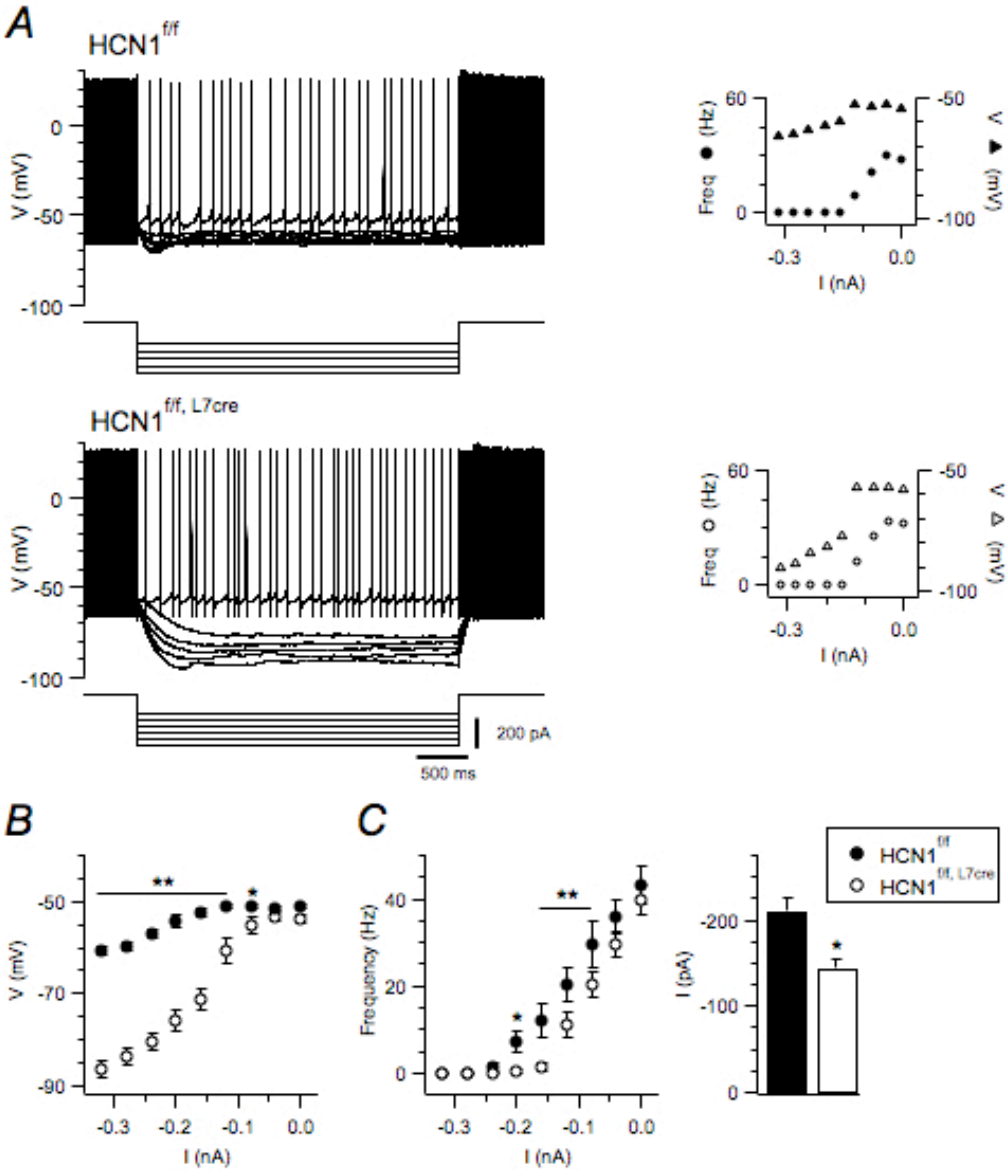
A, representative recordings of I_h from a wild-type Purkinje cell in the absence (left) or presence (right) of the I_h blocker ZD7288 (0.01 mM). Hyperpolarization to voltages between -55 mV and -120 mV in 5 mV increments from a holding potential of -50 mV (lower traces) activated a prominent I_h that was reduced by bath-applied ZD7288 (upper traces). *B,* tail currents measured upon return to -50 mV, plotted against the preceding test potential, confirm that ZD7288 reduced I_h in Purkinje cells from HCN1$^{f/f}$ mice (genotype $F_{1,6}$=149.03, P<0.0001; test potential $F_{13,78}$=119.49, P<0.0001; genotype X test potential $F_{13,78}$=62.76, P<0.0001, n=4 per group), but had no effect on tail currents measured from Purkinje cells from HCN1$^{f/f,L7cre}$ mice (genotype $F_{1,8}$=0.58, P=0.47; test potential $F_{13,104}$=2.94, P=0.001; genotype X test potential $F_{13,104}$=0.87, P=0.58, n=5 per group). Data are presented as mean ± s.e.m. *P < 0.01 HCN1$^{f/f}$ *vs* HCN1$^{f/f,L7cre}$, Fisher's PLSD.



Supplemental Figure 2. **HCN1 channels are not required for spontaneous firing of cerebellar Purkinje cells.**

A, representative membrane potential recordings from Purkinje cells in acute slices from HCN1$^{f/f}$ and HCN1$^{f/f,L7cre}$ mice showing that spontaneous activity was not compromised by HCN1 deletion. *B,* both HCN1$^{f/f}$ (n=13) and HCN1$^{f/f,L7cre}$ mice (n=17) showed regular and stable spontaneous firing. Spike frequency (t_{28}=1.17, *P*=0.25, Student's unpaired t-test) and mean inter-spike interval (t_{28}=1.4, *P*=0.17) measured intracellularly were comparable between groups. Similar results were obtained in cell attached recordings (t_{23}=0.26, *P*=0.8; t_{23}=0.19, *P*=0.85, n=9-16 per group). There were no differences between wild-type and knock-out mice in the variability of inter-spike interval (t_{28}=0.42, *P*=0.68), median membrane potential (t_{28}=2.6, *P*=0.01), action potential threshold (t_{28}=2.29,

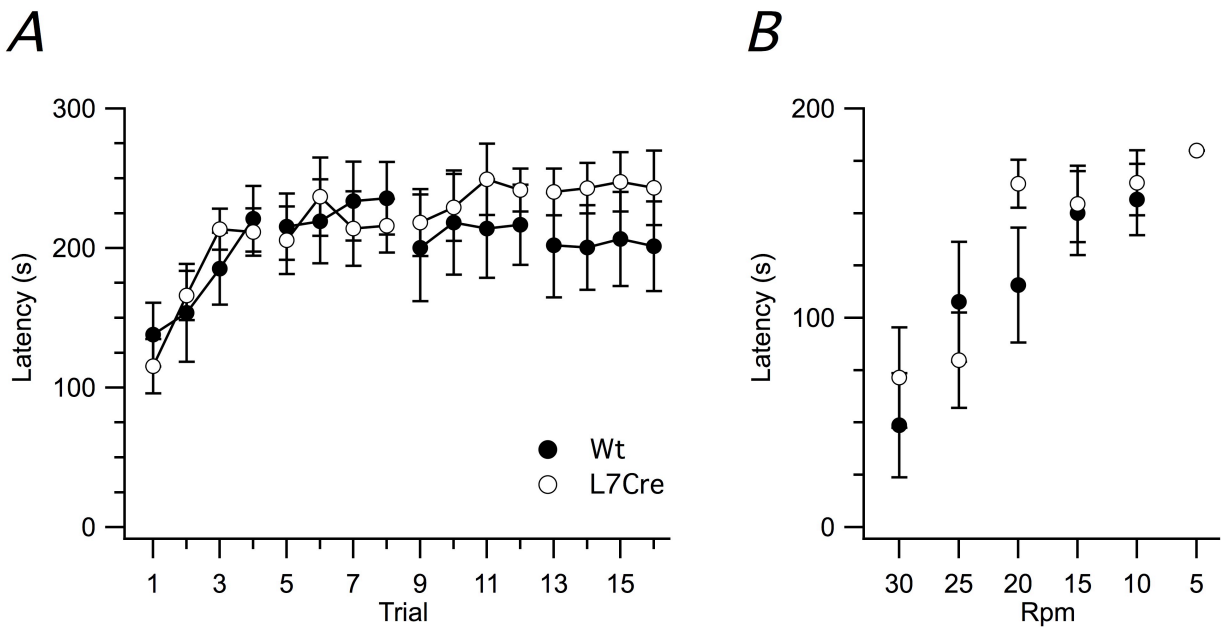
P=0.03), the 10%-90% rise time (t_{28}=0.43, *P*=0.67), half width (t_{28}=0.95, *P*=0.35) and peak depolarization of the action potential (t_{28}=0.98, *P*=0.33) or the peak amplitude of the after-hyperpolarization (t_{28}=2.3, *P*=0.03). Horizontal lines represent mean ± s.e.m. Circular markers represent individual cells. Level of significance was set at $P < 0.005$ by using Bonferroni's correction for multiple comparisons.



Supplemental Figure 3. HCN1 channels influence responses of Purkinje cells to negative current steps.

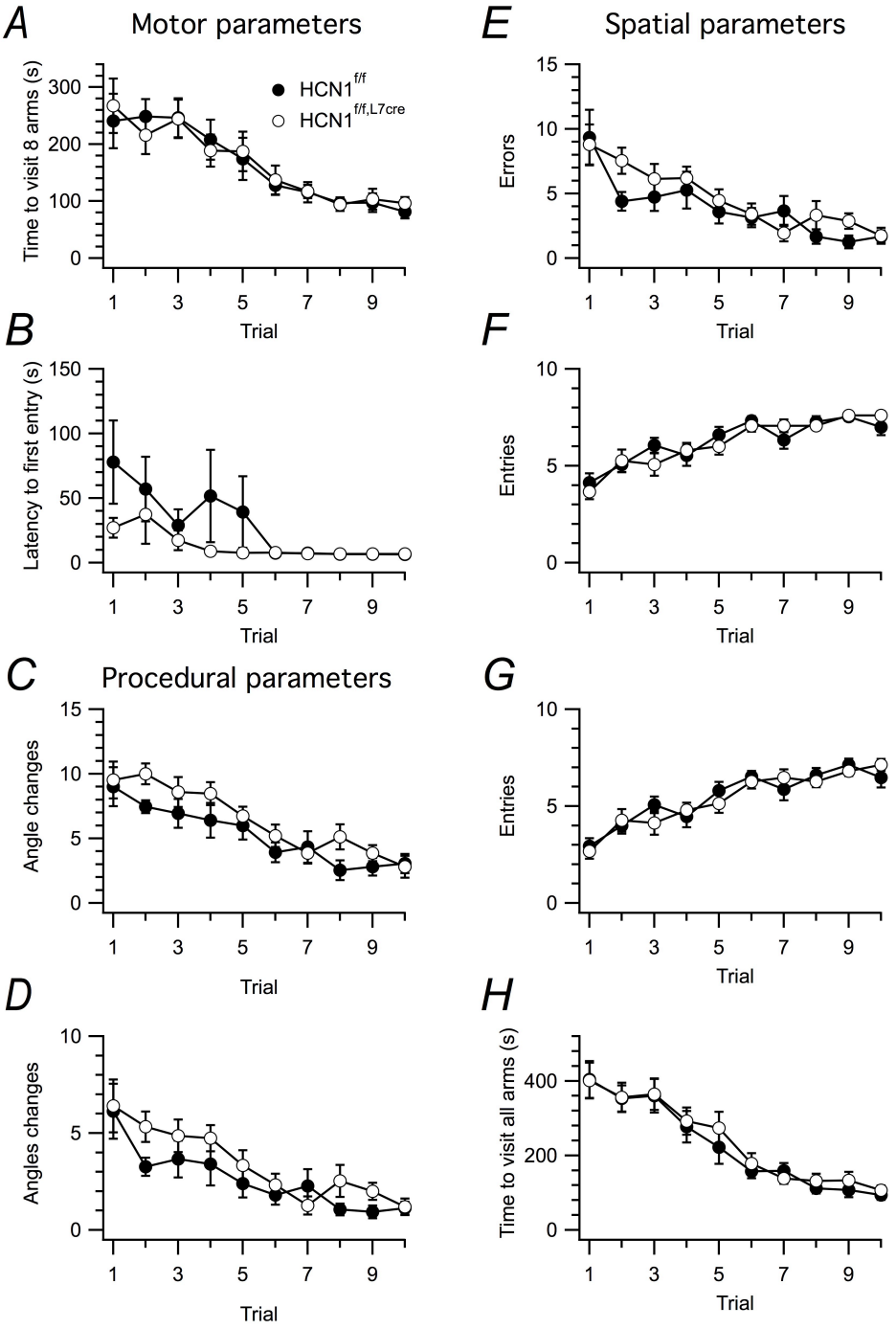
A, representative recordings of the membrane potential response of Purkinje cells from HCN1$^{f/f}$ and HCN1$^{f/f, L7cre}$ mice to a series of negative current steps of amplitude up to -320 pA in 40 pA increments. Graphs (right) plot the steady-state action potential frequency and modal membrane potential as a function of the amplitude of the current step. Because the modal membrane potential is dominated by the voltage trajectory during the interspike interval, it is relatively insensitive to spike frequency unless the injected current is sufficient to abolish spike firing. *B and C***,** mean steady-state modal membrane potential (*B*) and action potential frequency (*C*, left panel) plotted as a function of current step amplitude. The steady-state hyperpolarization in response to negative current steps was significantly increased in Purkinje cells from HCN1$^{f/f,L7cre}$ (n=10) compared to HCN1$^{f/f}$ mice (n=16) (genotype $F_{1,24}$=56.12, P<0.0001; current $F_{8,192}$=101.63, P<0.0001; genotype

X current $F_{8,192}$=34.9, P<0.0001, two-way ANOVA). The steady-state spike frequency was reduced with increasing current step amplitude in both $HCN1^{f/f}$ and $HCN1^{f/f,L7cre}$ mice (genotype $F_{1,24}$=4.2, P=0.05; current $F_{8,192}$=126.33, P<0.0001; genotype X current $F_{8,192}$=2.27, P=0.02, two-way ANOVA), but the minimal current required to abolish spontaneous firing was reduced in knock-out mice (*C*, right panel) (t_{24}=3.62, P=0.001, Student's unpaired t-test). Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.001 $HCN1^{f/f}$ *vs* $HCN1^{f/f,L7cre}$.



Supplemental Figure 4. Cre expression in Purkinje cells does not affect motor learning in the rotarod.

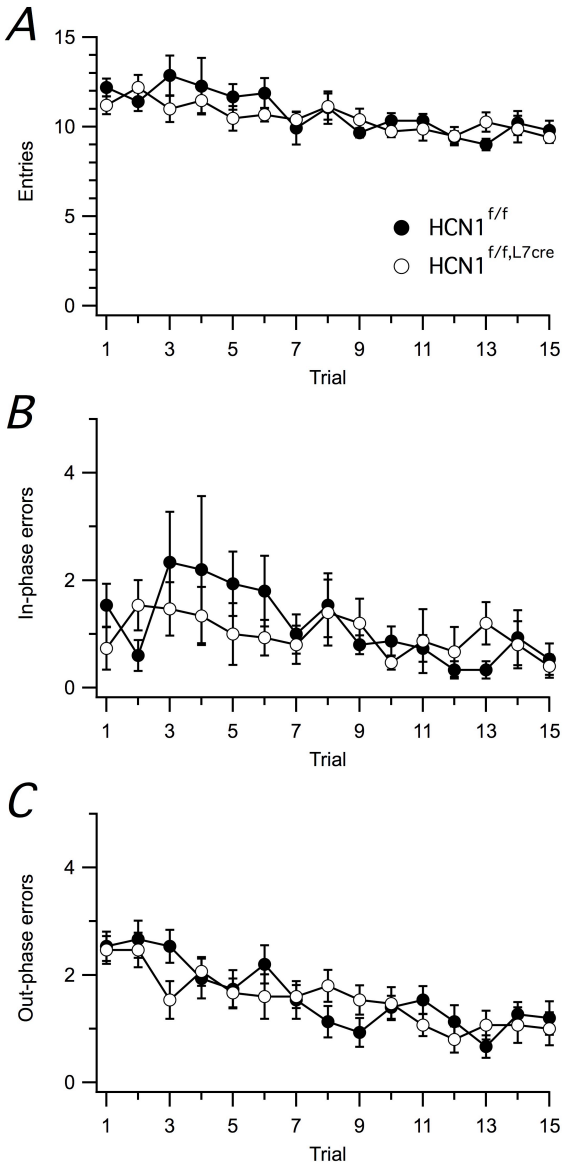
A, time to fall from a 3 cm wide accelerating rotarod is plotted as a function of training trial for mice expressing Cre under the control of the L7 promoter and wild-type littermates. The two groups similarly increased their performance across trials (genotype $F_{1,17}$=0.23, P=0.64; trial $F_{15,255}$=6.35, P<0.0001; genotype X trial $F_{15,255}$=1.11, P=0.35; n=9-10 per group, two-way ANOVA). *B,* L7Cre and wild-type mice did not show significant differences when tested with the rod moving at constant speeds (genotype $F_{1,17}$=0.29, P=0.6; rpm $F_{5,85}$=14.45, P<0.0001; genotype X rpm $F_{5,85}$=1.13, P=0.35; n=9-10 per group, two-way ANOVA). Data are presented as mean ± s.e.m.



Supplemental Figure 5. HCN1 channels in Purkinje cells are not required for spatial learning in the fully-baited version of the radial maze.

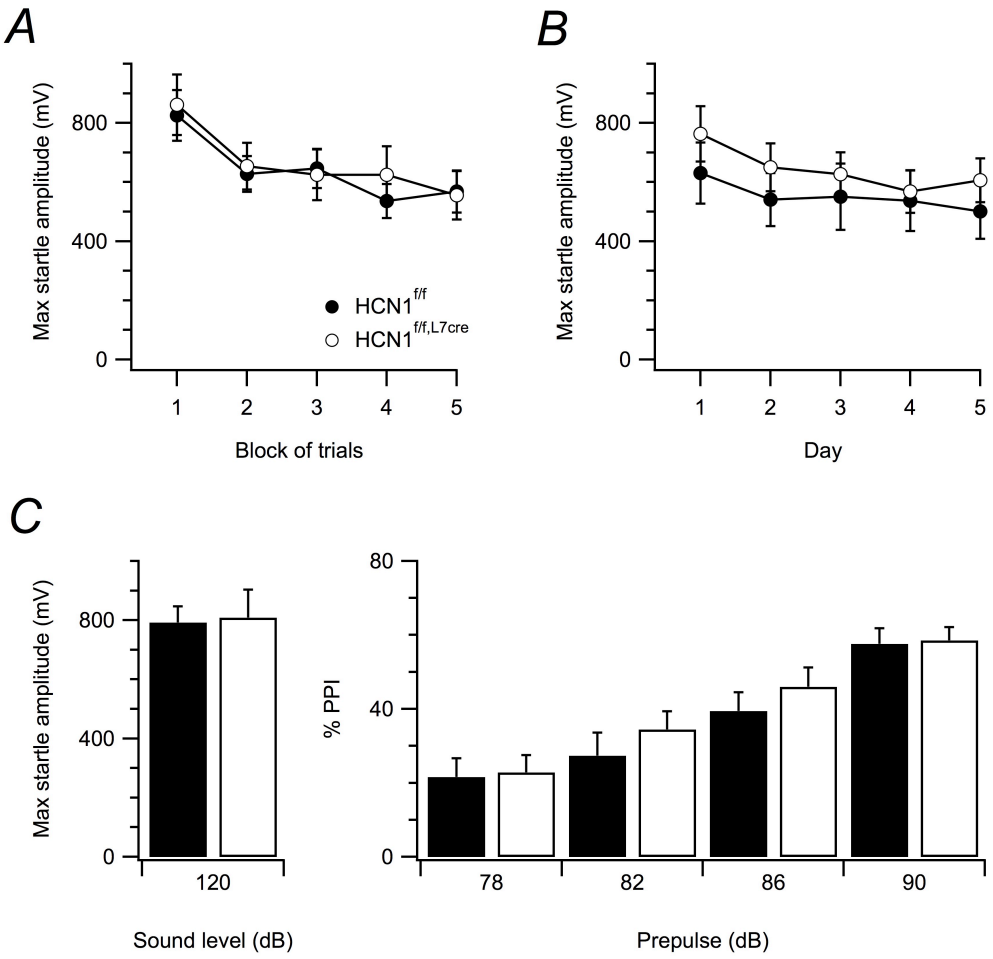
A and *B,* HCN1$^{f/f}$ and HCN1$^{f/f, L7cre}$ mice did not show differences in measures of locomotor activity in the radial maze (*A*, time to visit 8 arms, either right or wrong: genotype $F_{1,28}$=0.004, *P*=0.95; trial $F_{9,252}$=16.56, *P*<0.0001; genotype X trial $F_{9,252}$=0.29, *P*=0.98; *B*, latency to enter in the first arm: genotype $F_{1,28}$=1.88, *P*=0.18; trial $F_{9,252}$=3.45, *P*<0.0005; genotype X trial $F_{9,252}$=1.09, *P*=0.37). *C* and *D,* no differences were observed between wild-type and knock-out mice in parameters linked to procedural components of the task (*C*, angle change, defined as the number of angle changes performed in a trial: genotype $F_{1,28}$=2.85, *P*=0.1; trial $F_{9,252}$=13.97, *P*<0.0001; genotype X trial

$F_{9,252}$=0.72, *P*=0.69; *D*, strategy fixation, calculated as angle changes after an error was made: genotype $F_{1,28}$=2.66, *P*=0.11; trial $F_{9,252}$=10.23, *P*<0.0001; genotype X trial $F_{9,252}$=0.7, *P*=0.71). *E-H,* there were no differences between $HCN1^{f/f}$ and $HCN1^{f/f, L7cre}$ mice in spatial learning parameters (*E*, working memory errors: genotype $F_{1,28}$=0.25, *P*=0.27; trial $F_{9,252}$=11.48, *P*<0.0001; genotype X trial $F_{9,252}$=0.99, *P*=0.45; *F*, number of arms visited until the first wrong visit and including it: genotype $F_{1,28}$=0.06, *P*=0.8; trial $F_{9,252}$=21.31, *P*<0.0001; genotype X trial $F_{9,252}$=1.15, *P*=0.33; *G*, spatial span, defined as the longest sequence of correctly visited arms: genotype $F_{1,28}$=0.1, *P*=0.76; trial $F_{9,252}$=23.87, *P*<0.0001; genotype X trial $F_{9,252}$=0.92, *P*=0.5; *H*, total time needed to visit all arms at least once: genotype $F_{1,28}$=0.2, *P*=0.66; trial $F_{9,252}$=34.32, *P*<0.0001; genotype X trial $F_{9,252}$=0.25, *P*=0.99). Data are presented as mean ± s.e.m. and analyzed by two-way ANOVA, n=15 per group.



Supplemental Figure 6. HCN1 channels in Purkinje cells are not required for spatial learning in the delayed version on the radial maze.

A-C, performance in the delayed version of the radial maze plotted as a function of training trial. Both HCN1$^{f/f}$ and HCN1$^{f/f,L7cre}$ mice significantly improved their performance across trials indicating that spatial memory was not affected by the deletion (*A*, number of entries before all arms were visited: genotype $F_{1,28}$=0.64, *P*=0.43; trial $F_{14,392}$=3.98, *P*<0.0001; genotype X trial $F_{14,392}$=0.9, *P*=0.55; *B*, in-phase working memory errors defined as re-entries into an arm already visited in the same phase: genotype $F_{1,28}$=0.44, *P*=0.51; trial $F_{14,392}$=1.57, *P*=0.08; genotype X trial $F_{14,392}$=0.8, *P*=0.67; *C*, out-phase working memory errors, defined as re-entries into an arm visited during the sample phase: genotype $F_{1,28}$=0.22, *P*=0.65; trial $F_{14,392}$=6.98, *P*<0.0001; genotype X trial $F_{14,392}$=1.23, *P*=0.25). Data are presented as mean ± s.e.m. and analyzed by two-way ANOVA, n=15 per group.



Supplemental Figure 7. HCN1 deletion in Purkinje cells does not affect habituation and prepulse inhibition of the acoustic startle response.

A, both HCN1$^{f/f}$ and HCN1$^{f/f,L7cre}$ mice showed a progressive significant decrease in the amplitude of the startle response. No differences were observed between the two groups (genotype $F_{1,46}$=0.05, *P*=0.82; day $F_{4,184}$=17.549, *P*<0.0001; genotype X day $F_{4,184}$=0.67, *P*=0.61; n=24 per group, two-way ANOVA). Results are presented as mean of blocks of 20 trials. *B,* long term habituation of the startle response was measured testing mice as described in *A*, for 5 days. Both wild-type and knock-out mice showed significant long term habituation. No differences were observed in the amplitude of the response between the groups (genotype $F_{1,28}$=0.56, *P*=0.46; day $F_{4,112}$=5.38, *P*=0.0005; genotype X day $F_{4,112}$=0.58, *P*=0.67; n=11-19 per group, two-way ANOVA). Results are presented as mean of 100 trials for each day. *C,* there were no differences between HCN1$^{f/f}$ and HCN1$^{f/f,L7cre}$ mice in the response to the startle stimulus (left, t_{28}=0.15, *P*=0.88, Student's unpaired t-test) and in the prepulse-induced reduction of the startle response (right, genotype $F_{1,28}$=0.92, *P*=0.35; pre-pulse $F_{4,112}$=109.14, *P*<0.0001; genotype X pre-pulse $F_{4,112}$=2.01, *P*=0.1; n=15 per group, two-way ANOVA). Data are presented as mean ± s.e.m.

Supplemental Data. Complete statistical analysis for Figure 4.

D, Gain 0.2 Hz: genotype $F_{1,23}$=0.34, *P*=0.56, trial $F_{1,23}$=115.33, *P*<0.0001, genotype X trial $F_{1,23}$=0.18, *P*=0.67. Gain 0.5 Hz: genotype $F_{1,24}$=0.58, *P*=0.46, trial $F_{1,24}$=189.47, *P*<0.0001, genotype X trial $F_{1,24}$=0.07, *P*=0.79. Gain 1 Hz: genotype $F_{1,23}$=1.09, *P*=0.31, trial $F_{1,23}$=117.39, *P*<0.0001, genotype X trial $F_{1,23}$=0.31, *P*=0.58. Gain 2 Hz: genotype $F_{1,22}$=0.3, *P*=0.59, trial $F_{1,22}$=139.78, *P*<0.0001, genotype X trial $F_{1,22}$=0.39, *P*=0.54. Phase 0.2 Hz: genotype $F_{1,23}$=1.85, *P*=0.19, trial $F_{1,23}$=13.22, *P*=0.001, genotype X trial $F_{1,23}$=13.89, *P*=0.001. Phase 0.5 Hz: genotype $F_{1,24}$=0.001, *P*=0.98, trial $F_{1,24}$=1.1, *P*=0.3, genotype X trial $F_{1,24}$=2.49, *P*=0.13. Phase 1 Hz: genotype $F_{1,23}$=0.1, *P*=0.75, trial $F_{1,23}$=2.23, *P*=0.15, genotype X trial $F_{1,23}$=0.21, *P*=0.65. Phase 2 Hz: genotype $F_{1,22}$=0.82, *P*=0.37, trial $F_{1,22}$=4.79, *P*=0.04, genotype X trial $F_{1,22}$=0.15, *P*=0.7.

E, Gain: genotype $F_{1,22}$=0.006, *P*=0.94, time $F_{4,88}$=93.41, *P*<0.0001, genotype X time $F_{4,88}$=0.86, *P*=0.49. Phase: genotype $F_{1,22}$=1.13, *P*=0.3, time $F_{4,88}$=7.13, *P*<0.0001, genotype X time $F_{4,88}$=3.33, *P*=0.01.

F, Gain: genotype $F_{1,23}$=0.04, *P*=0.84, day $F_{4,92}$=224.3, *P*<0.0001, genotype X day $F_{4,92}$=1.78, *P*=0.14. Phase: genotype $F_{1,23}$=4.66, *P*=0.04, day $F_{4,92}$=7.41, *P*<0.0001, genotype X day $F_{4,92}$=0.78, *P*=0.54.

G, Gain 0.2 Hz: genotype $F_{1,18}$=0.06, *P*=0.8, day $F_{4,72}$=27.18, *P*<0.0001, genotype X day $F_{4,72}$=3.05, *P*=0.02. Gain 0.5 Hz: genotype $F_{1,21}$=0.13, *P*=0.72, day $F_{4,84}$=40.33, *P*<0.0001, genotype X day $F_{4,84}$=0.88, *P*=0.48. Phase 0.2 Hz: genotype $F_{1,18}$=2.17, *P*=0.16, day $F_{4,72}$=11.02, *P*<0.0001, genotype X day $F_{4,72}$=3.67, *P*=0.009. Phase 0.5 Hz: genotype $F_{1,21}$=1.27, *P*=0.27, day $F_{4,84}$=4.22, *P*=0.004, genotype X day $F_{4,84}$=3, *P*=0.03; n=11-12 per group.

Data were analyzed by two-way ANOVA.