RESEARCH ARTICLE

# Figures and figure supplements

Cerebellum encodes and influences the initiation, performance, and termination of discontinuous movements in mice

**Michael A Gaffield *et al***

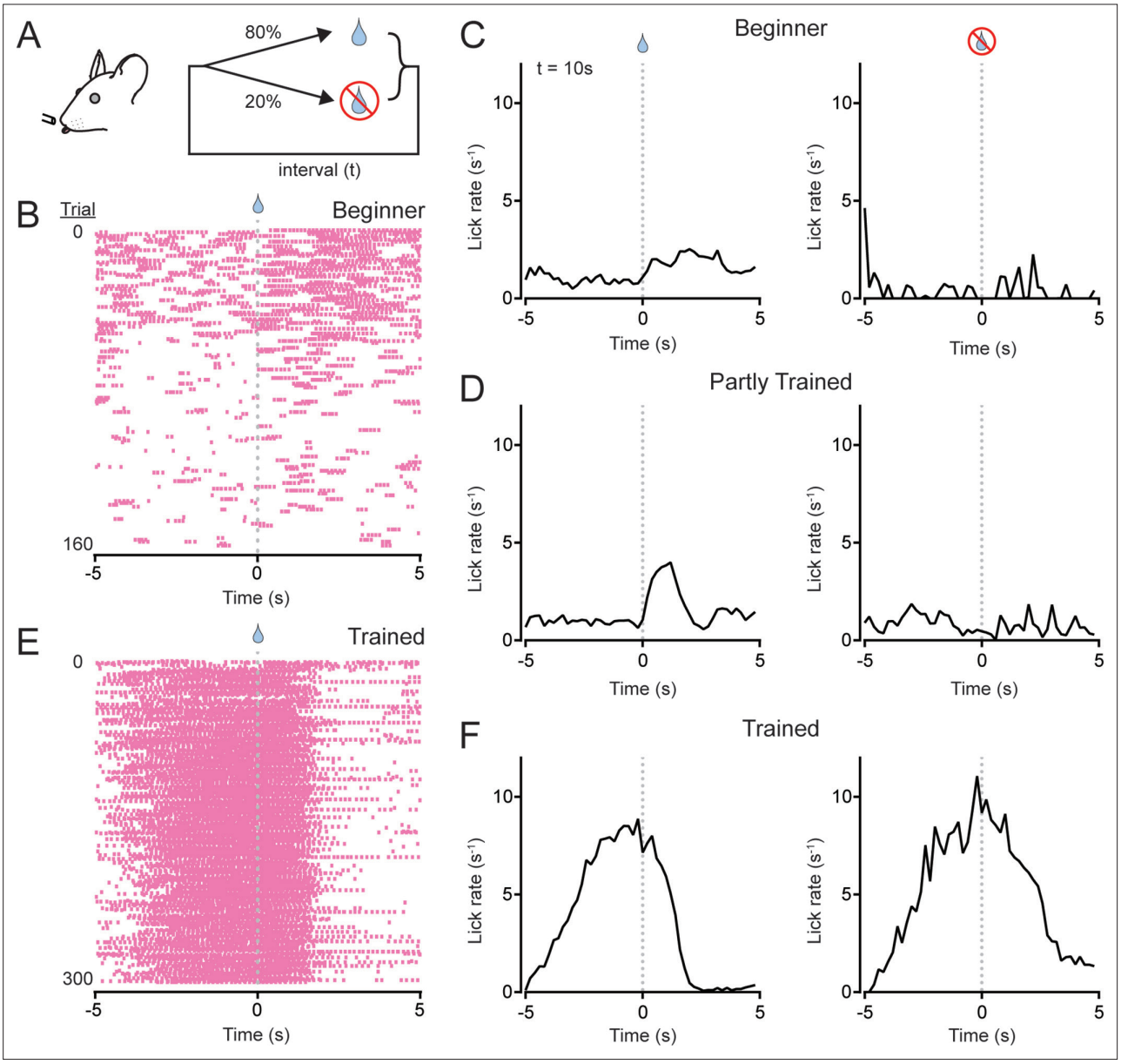


**Figure 1.** An interval timing task to assess the role of the cerebellum in organizing periodic, discontinuous movement. (**A**) Schematic diagram of the task. Mice were trained to lick for water rewards delivered at a regular time interval (*t*). Water was allocated in most trials and withheld in the others. (**B**) Lick patterns of a beginner mouse during an early training session. Licks are indicated by pink tic marks; water was allocated at the time indicated by the droplet (*t* = 10 s). (**C**) Trial-averaged lick rates for the beginner mouse with session trials (159 total) separated based on whether water was allocated (left; rewarded) or withheld (right; unrewarded). (**D**) Same as panel C but after the mouse received additional sessions of training (216 total trials). (**E**) Lick patterns over the course of a session after full training (same mouse as in panels B and C). (**F**) Trial-averaged lick rates of the fully trained animal during an individual session (300 total trials).

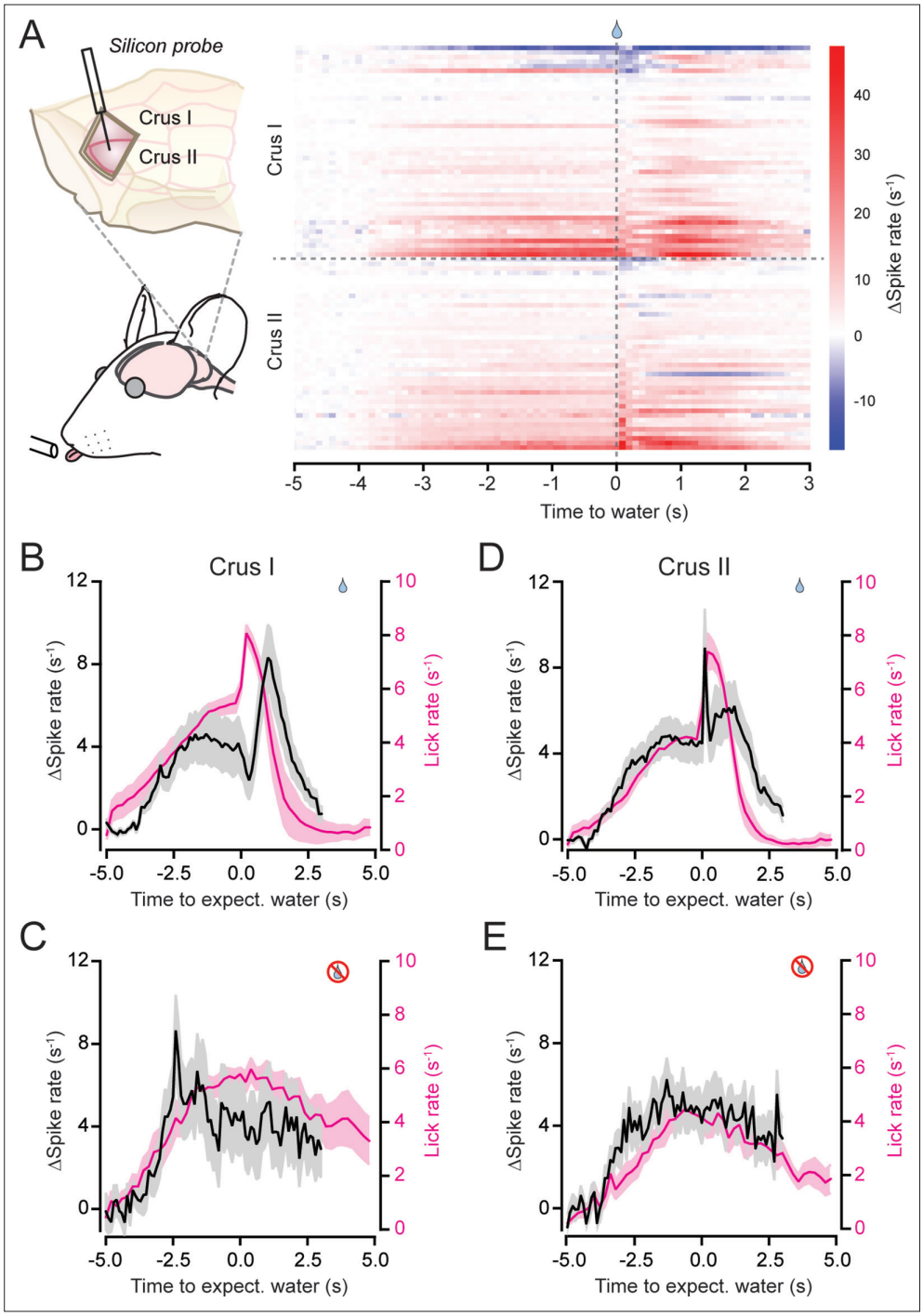


**Figure 2.** Modulation of Purkinje cell (PC) simple spiking during the performance of discontinuous movement. (**A**) Left: electrophysiological activity was recorded from PCs using silicon probes targeting either the left Crus I or II through a large craniotomy. Right: changes in simple spiking firing, relative to non-licking baseline, for all PCs during water-rewarded trials. Data are separated by lobule and sorted based on average activity-level changes within ±200 ms of water allocation ($n$ = 47 Crus I PCs from 6 mice; $n$ = 42 Crus II PCs from 5 mice). (**B**) The mean change in simple spike rate (black), relative to baseline, for Crus I PCs during water-rewarded trials. The corresponding trial-averaged lick rate is also shown (pink). (**C**) Same as panel B but for trials in which water was withheld. (**D, E**) Same as panels B and C but for Crus II PCs.

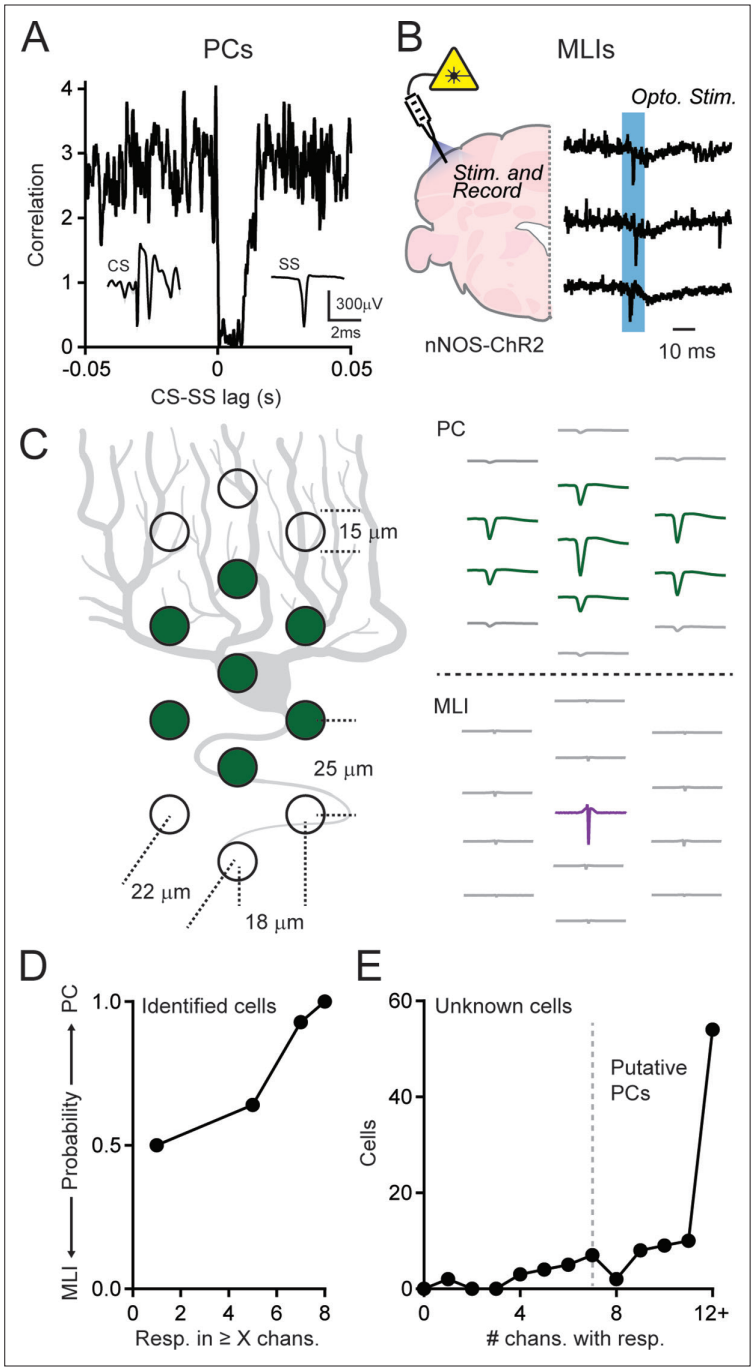


**Figure 2—figure supplement 1.** Identification of Purkinje cells (PCs) in silicon probe data. (**A**) PCs were unambiguously identified by the appearance of both complex spikes (left inset) and simple spikes (right inset) in the unit recordings. The correlation plot of these two spike types revealed the characteristic complex-spike-induced pause in simple spike firing. Data are from an individual PC. (**B**) MLIs were identified using an opto-tag strategy. Specifically, ChR2-expressing MLIs showed short-latency spiking responses to brief optogenetic stimuli. Example traces show evoked activity in three different MLIs from the same mouse with the photostimulus period indicated in blue. (**C**) Left: illustration of the arrangement of electrode pads on the silicon probe overlaid on a prototypic PC. Top right: mean spikes recorded in each electrode channel for an example PC. Bottom right: mean spikes recorded in each electrode channel for an example MLI. The purple trace is the only channel with a response. (**D**) Plot of the probability that cells whose spikes were observed in at least X channels had a confirmed identity as either a PC ($n$ = 23 cells) or an MLI ($n$ = 25 cells, total of 3 mice). (**E**) Histogram of all potential PCs, based on simple spike firing characteristics ($n$ = 104), and the number of channels with detectable spikes for each cell. Based on the analysis in panel D, all cells with spikes in seven or more channels were classified as PCs.

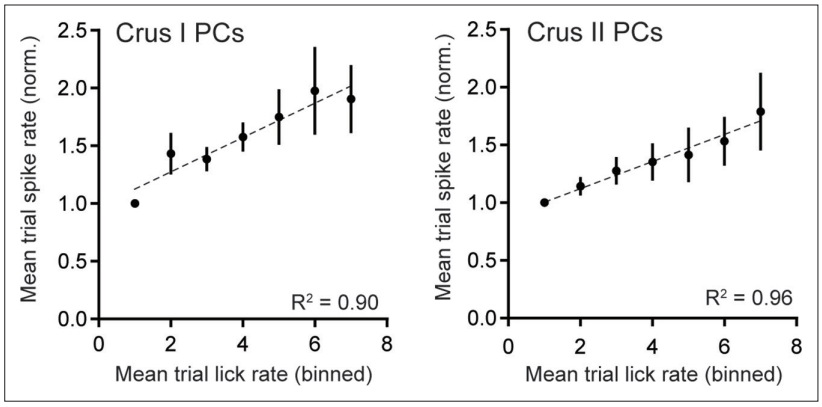


**Figure 2—figure supplement 2.** Movement-related Purkinje cell (PC) simple spiking activity. Relationship between spiking activity and movement for Crus I (left) and Crus II (right) PCs. Simple spike firing rates are binned based on the corresponding mean lick rate during the trial. Dashed line is the linear correlation.

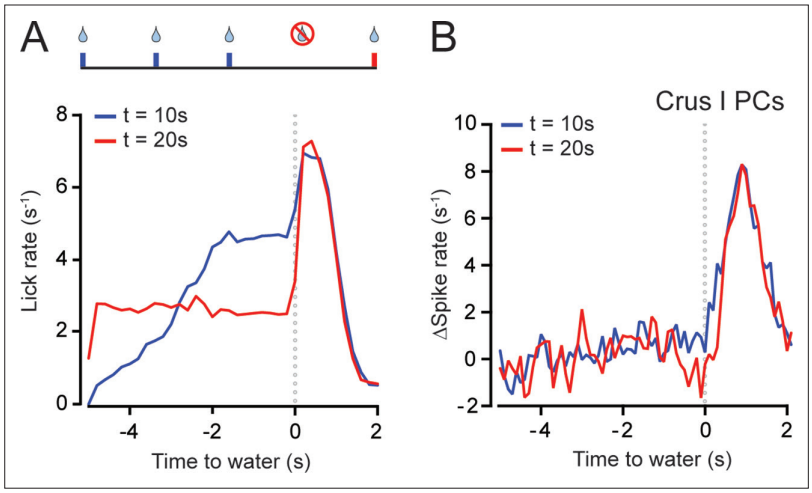


**Figure 2—figure supplement 3.** Purkinje cell (PC) simple spiking activity does not change in response to unexpected intervals. (**A**) Top: licking trials were separated dependent on whether water was allocated at the expected regular interval (*t* = 10 s; blue tics) or at an unexpectedly prolonged interval resulting from water omission on the immediately preceding trial (*t* = 20 s; red tic). Below: average licking activity across mice for the two trial types. (**B**) The corresponding change in simple spiking activity in Crus I PCs to the two trial types, aligned to the time point of water rewards.

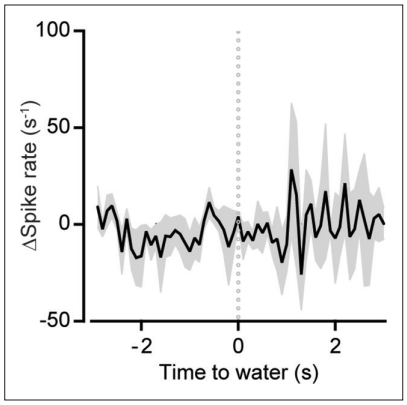


**Figure 2—figure supplement 4.** Lack of nonmotor Purkinje cell (PC) simple spiking activity in nonmovement trials. Average simple spiking activity in Crus I and II PCs for water allocation trials in which the mice did not elicit licks (*n* = 34 trials from 9 mice).

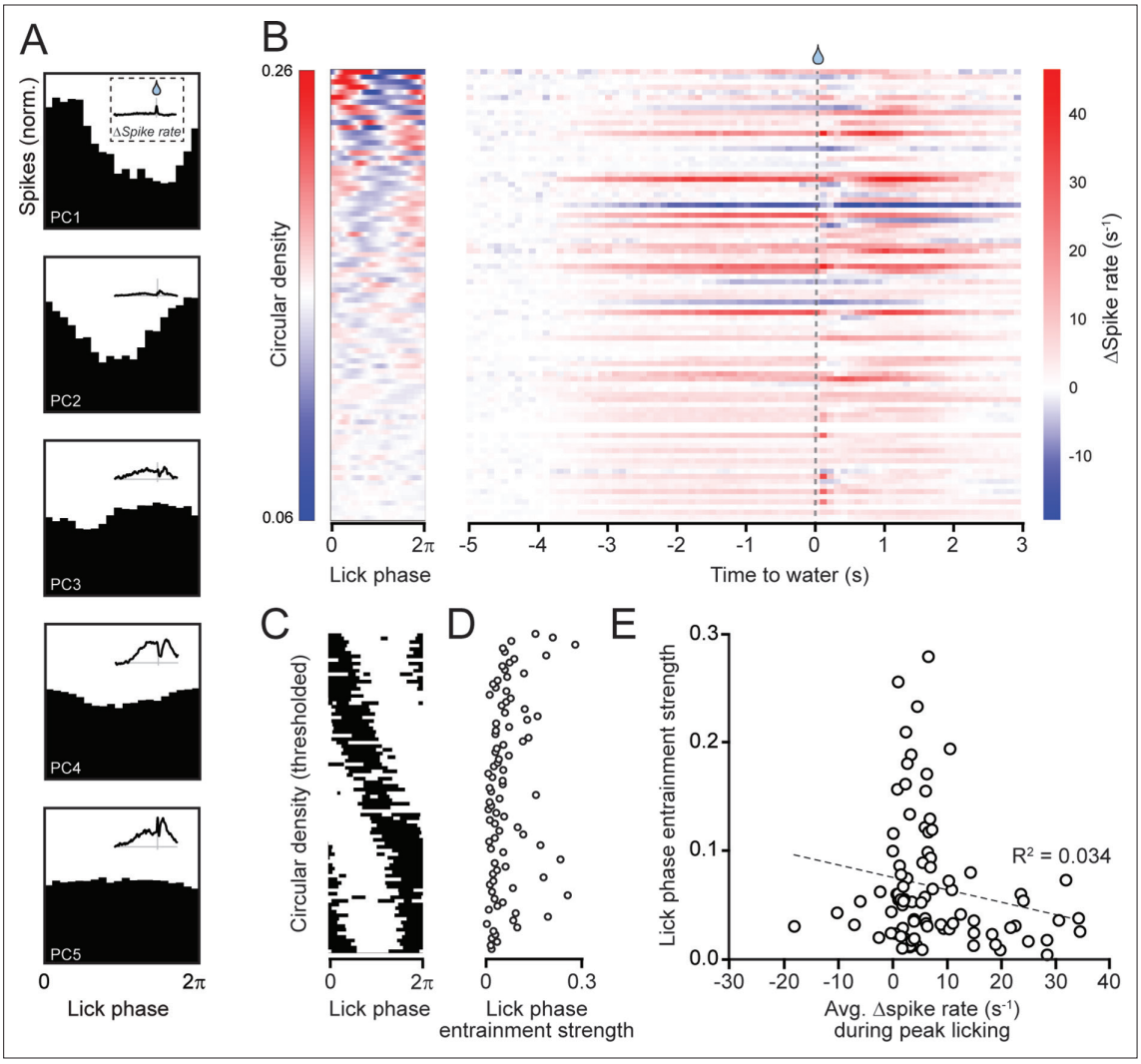


**Figure 3.** Entrainment of Purkinje cell (PC) simple spiking to the licking rhythm. (**A**) In spike histograms from five example PCs, lick phases sampled at spike times show varying levels of modulation. Contact between the tongue and the water port is defined to be 0 = 2*π*. The upper insets show the smoothed firing rate profiles of the same PC over the entire trial epoch, aligned to the time point of expected water allocation. (**B**) Probability density for spikes over the lick cycle (left) and the change in firing rate over the entire trial (right; reordered from ***Figure 2B***). Each row corresponds to a single PC and is sorted by the strength of entrainment to licking, defined as the mean resultant length. (**C**) Thresholded probability density for spikes over the lick cycle. Black regions indicate phases at which the density exceeds 1.02/2*π*. Rows are sorted by the entrainment phase, defined as the angle of the mean resultant. (**D**) Entrainment strength for all PCs, sorted as in C. (**E**) The relationship between firing rate modulation following water delivery and the strength of entrainment to the licking rhythm for individual PCs. The change in simple spike firing rate was averaged over the 2 s following water delivery. Dashed line is the linear correlation. Note, one outlier PC is off scale in panels D and E.

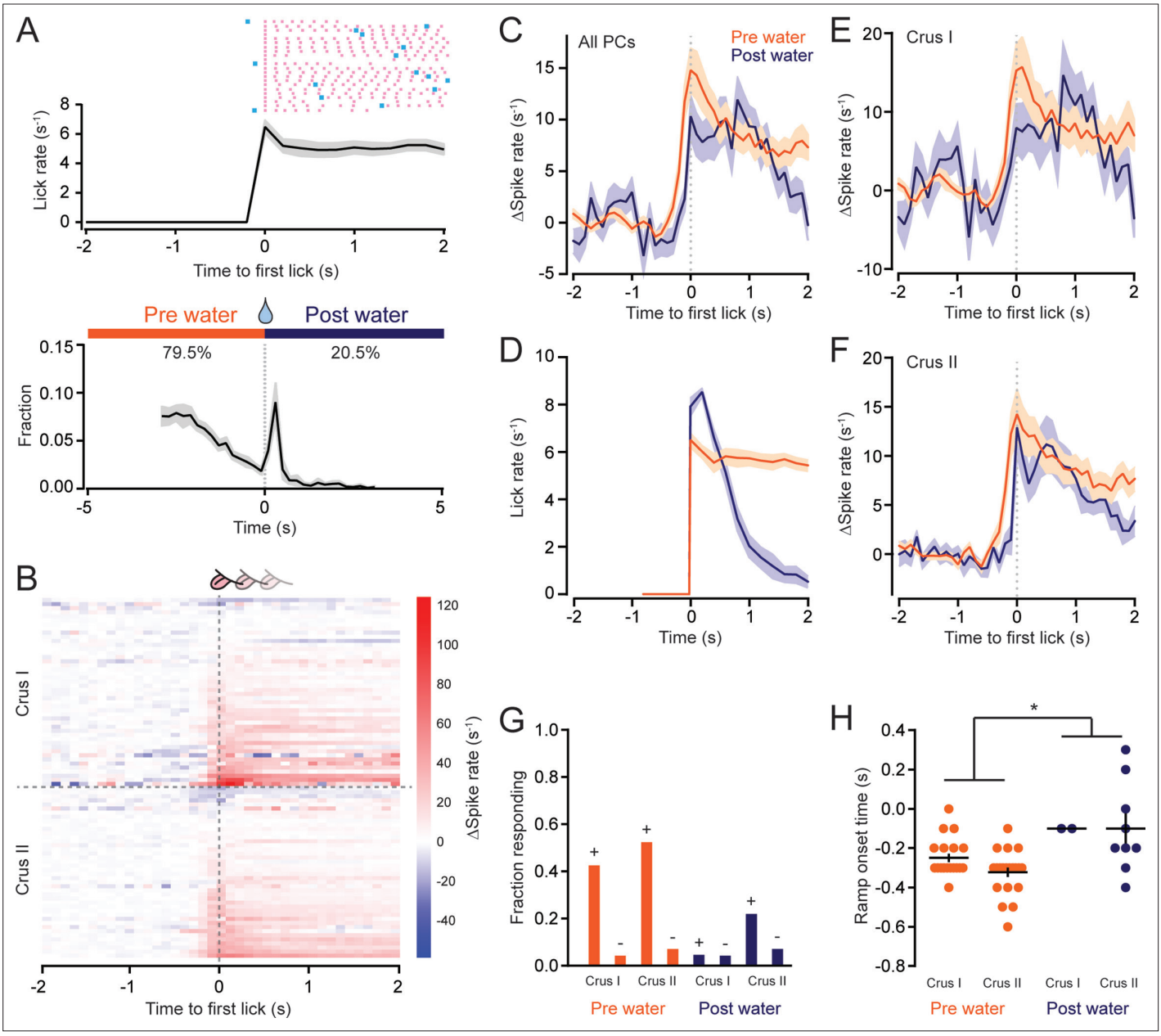


**Figure 4.** Modulation of Purkinje cell (PC) simple spiking during the initiation of discontinuous movement. (**A**) Top: plot of the mean lick rate aligned to the time point of the first lick in each water-rewarded trial bout ($n$ = 3164 trials of well-isolated licking bouts from 11 mice). The lick patterns for example trials of an individual mouse are also shown with licks indicated by pink tic marks and the time of water allocation indicated in blue. Bottom: histogram of lick-bout initiation times relative to water allocation. For most trials, mice began exploratory licking prior to water delivery (pre water). However, in the remaining trials, mice refrained from licking until after water allocation (post water), which immediately triggered a rapid increase in lick-bout initiations to consume the dispensed droplet. (**B**) The change in simple spiking activity relative to baseline for individual PCs sorted based on their average activity levels within ±200 ms of the first lick in each water-rewarded trial bout. (**C**) Trial-averaged change in simple spike activity for PCs, separated depending on whether mice initiated lick bouts before or after water allocation (pre and post water, respectively). Note the ramps in activity prior to licking. (**D**) Licking rates for bouts separated whether licking began before or after water allocation. (**E, F**) Same as panel C expect for Crus I (panel E) or Crus II (panel F) PCs. (G) Fraction of individual Crus I and II PCs with activity profiles that were either positively (+) or negatively (−) modulated around the time of lick-bout initiation, separated depending on whether licking began before or after water allocation (pre and post water, respectively). (**H**) Comparison of the onset times of activity ramping, relative to the first lick in trial bouts, for PCs whose activity positively modulated around lick-bout initiation. Data from each lobule were grouped together for statistical comparison (pre water: $n$ = 42 PCs from 10 mice; post water: $n$ = 11 PCs from 7 mice). Asterisk indicates significance (p = 0.0129, Student's $t$-test). See also ***Figure 4—source data 1***.

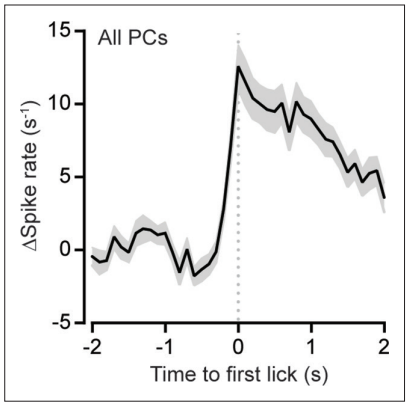


**Figure 4—figure supplement 1.** Purkinje cell (PC) simple spiking firing at lick-bout initiation. Plot of the average change in PC simple spike firing, relative to nonlicking baseline, aligned to the first lick in well-isolated bouts.

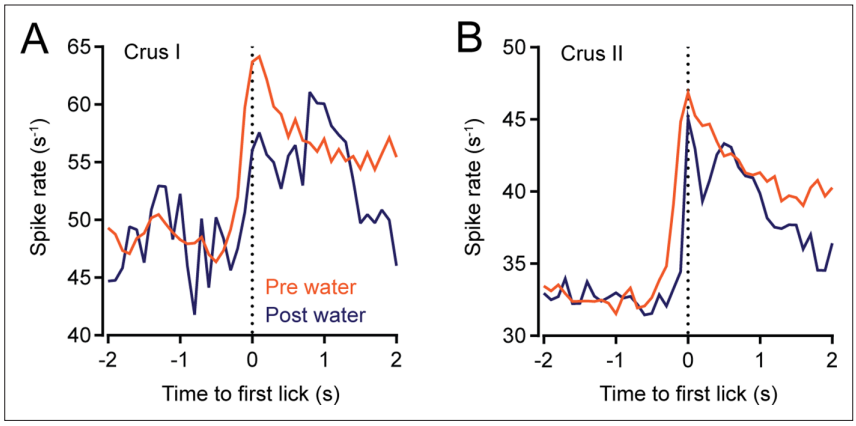


**Figure 4—figure supplement 2.** Simple spike firing rates of Purkinje cells (PCs) at lick-bout initiation. Same as ***Figure 4*** panels E and F but with simple spike firing for Crus I (**A**) and Crus II (**B**) PCs expressed as the absolute average firing rate rather than the difference relative to the nonlicking baseline.

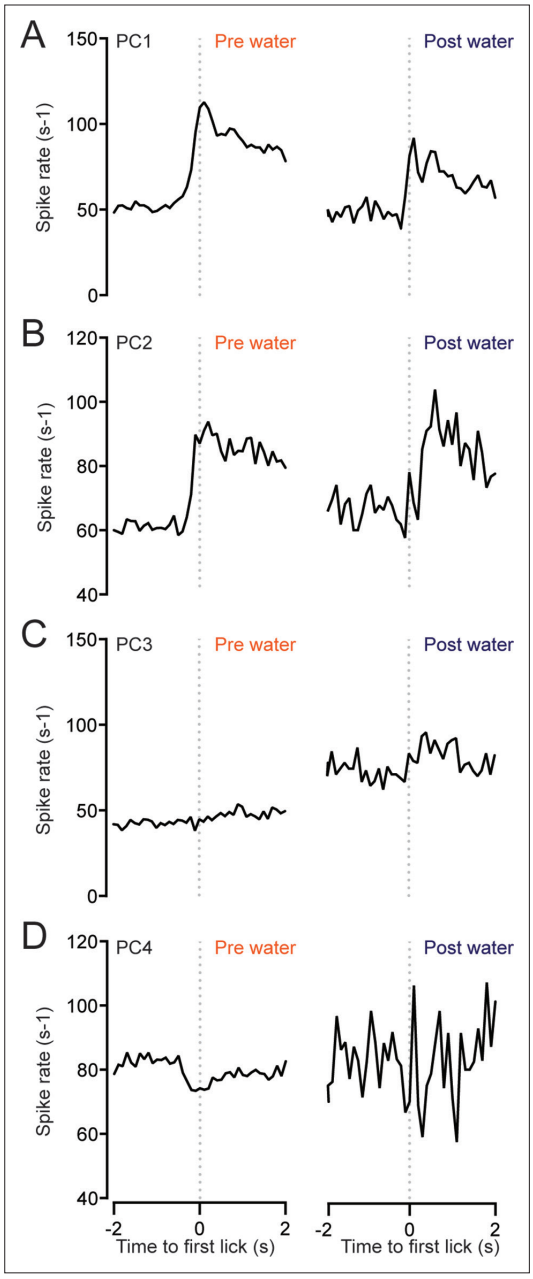


**Figure 4—figure supplement 3.** Simple spiking profiles of representative Purkinje cells (PCs) to lick-bout initiation. (**A–D**) Individual PCs (PC1–PC4) were sorted based on how their activity changed at lick-bout initiation. Averaged simple spiking patterns are shown for trials where licking began either prior to or after the time of water delivery (defined by the activity profile at the time window of −1 to 0.5 s; see Materials and methods). PC1 and PC2 (panels **A** and **B**) both had positively modulating simple spiking with ramping activity evident when licking anticipated water delivery in contrast to licking that was reactive to water allocation (pre and post water, respectively). PC3 (panel **C**) was unchanged during anticipatory licking but positively modulated during reactive licking, though

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the timing of the response aligned with the onset of licking. PC4 (panel **D**) negatively modulated during anticipatory licking but was unchanged during reactive licking.

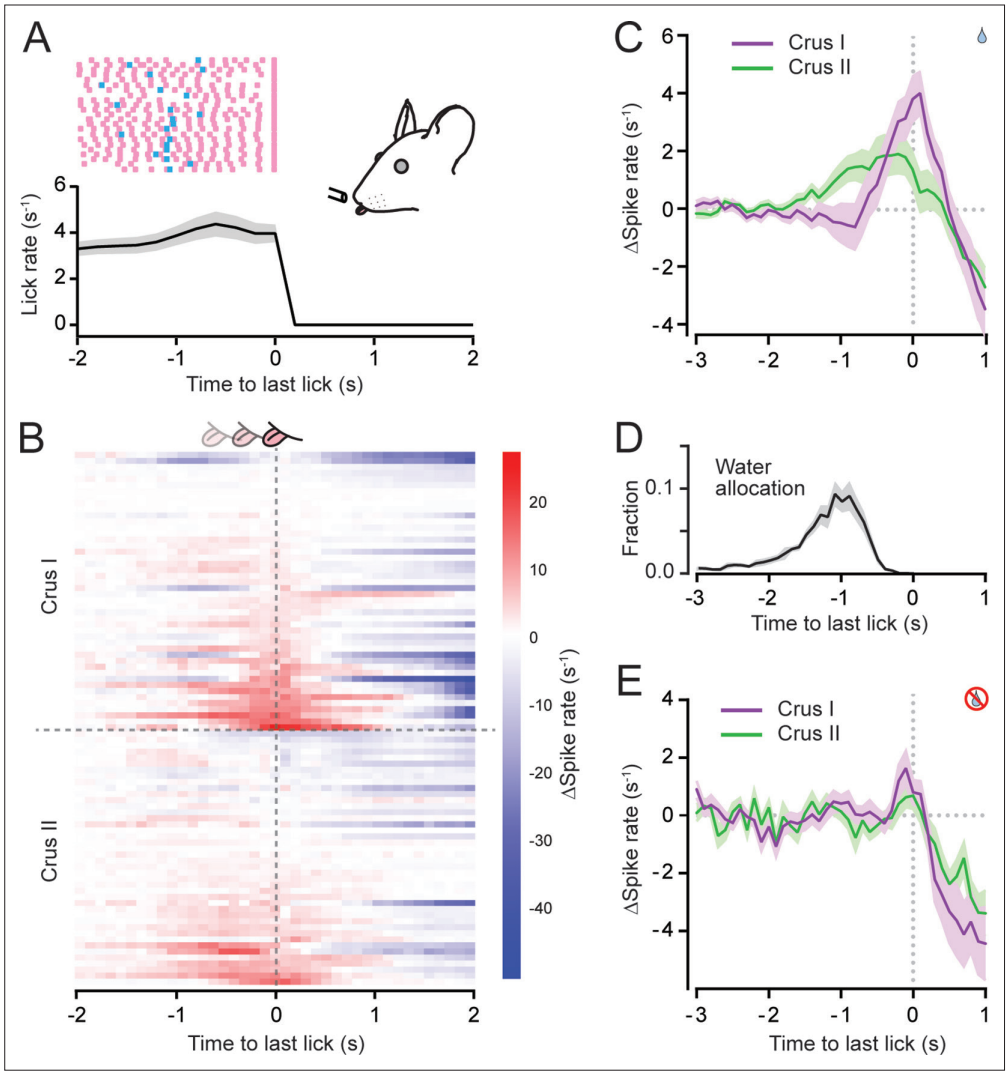


**Figure 5.** Modulation of Purkinje cell (PC) simple spiking during the termination of discontinuous movement. (**A**) Plot of mean lick rate aligned to the last lick in water-rewarded trial bouts (*n* = 2846 trials from 11 mice). Also shown are lick patterns of example trials for an individual mouse with licks indicated by pink tick marks and the timing of water allocation in blue. (**B**) Change in simple spike activity for individual PCs sorted based on their average activity levels within ±200 ms of the last lick in each water-rewarded trial bout. Responses were baselined to the lick-related activity in the seconds prior to the last lick. (**C**) Trial-averaged change in simple spike activity for Crus I (*n* = 47 from 6 mice) and Crus II (*n* = 42 from 5 mice) PCs aligned to the time of the last lick in water-rewarded trials. (**D**) Distribution of the timing of water allocation aligned to the point of the last lick in rewarded trial bouts (same trials as in panel A). (**E**) Same as panel C but for unrewarded trials (*n* = 44 Crus I PCs from 6 mice; *n* = 38 Crus II PCs from 5 mice).

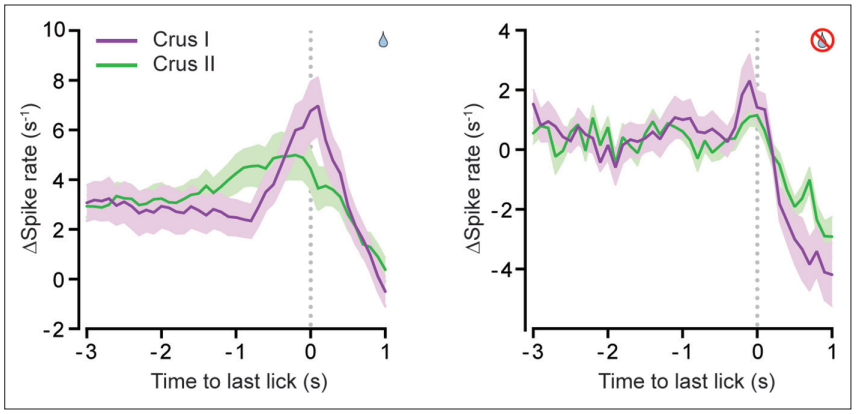


**Figure 5—figure supplement 1.** Last-lick-related Purkinje cell (PC) activity. Same as ***Figure 5*** panels C (left) and E (right) except that baseline was determined from all nonlicking periods during the recording.

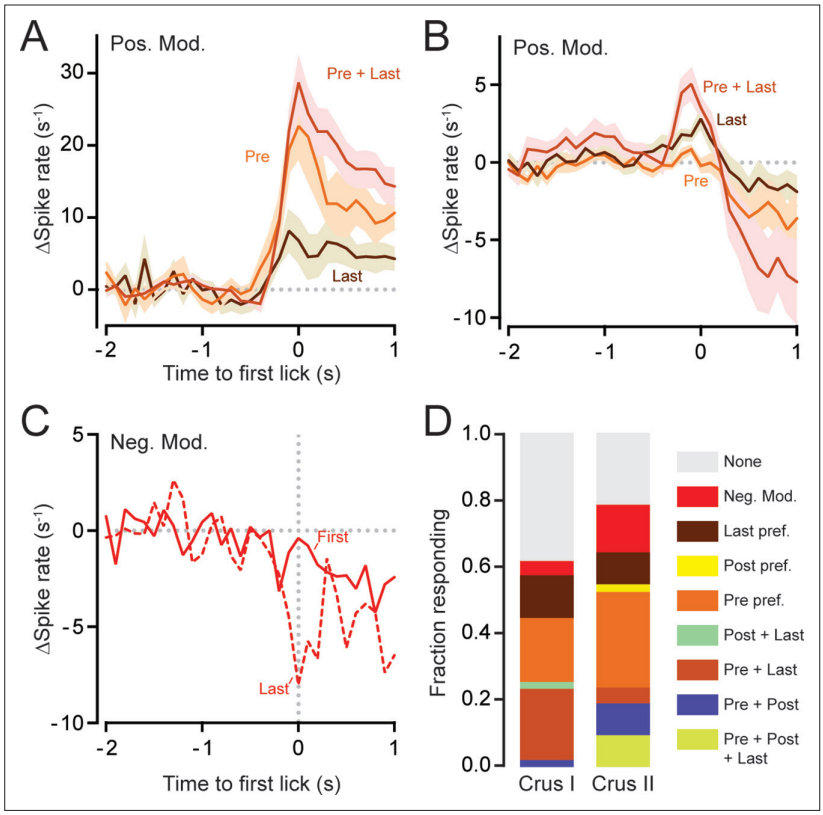


**Figure 5—figure supplement 2.** Task-feature tuning of individual Purkinje cells (PCs). (**A**) Trial-averaged activity profiles of PCs grouped based on the preference of their simple spiking profile for specific motor events during the task. PCs characterized as having a biased increase in firing to the first lick of exploratory bouts (Pre, *n* = 21 PCs from 9 mice) or to both the first licks of exploratory bouts and the last licks (Pre + Last, *n* = 16 PCs from 5 mice) showed a robust change at lick-bout initiation. By contrast, PCs characterized as preferentially responding only to the last lick in trial bouts (Last, *n* = 5 PCs from 3 mice) had a much smaller increase in simple spiking at lick-bout initiation. (**B**) Same PCs as panel A but during the time around the last lick in trial bouts. For last-lick-preferring PCs, as well as those that responded to both the first and last licks, there was a robust increase in firing at lick-bout termination. However, PCs with biased firing for only the first lick in exploratory bouts did not show any activity changes around lick-bout termination. (**C**) Trial-averaged simple spiking profiles of PCs characterized as having negatively modulating responses during first licks (solid line, *n* = 7 PCs from 6 mice) or last licks (dashed line, *n* = 5 PCs from 3 mice) during trial bouts. Standard error of the mean (SEM) ranges are removed for clarity. (**D**) Summary of tuning profiles for Crus I and II PCs (*n* = 47 and 42, respectively). PCs with a decrease in firing (Neg. Mod.) during either the first or last licks in trial bouts were considered as a single group as well as those without any discernable activity tuning (None).

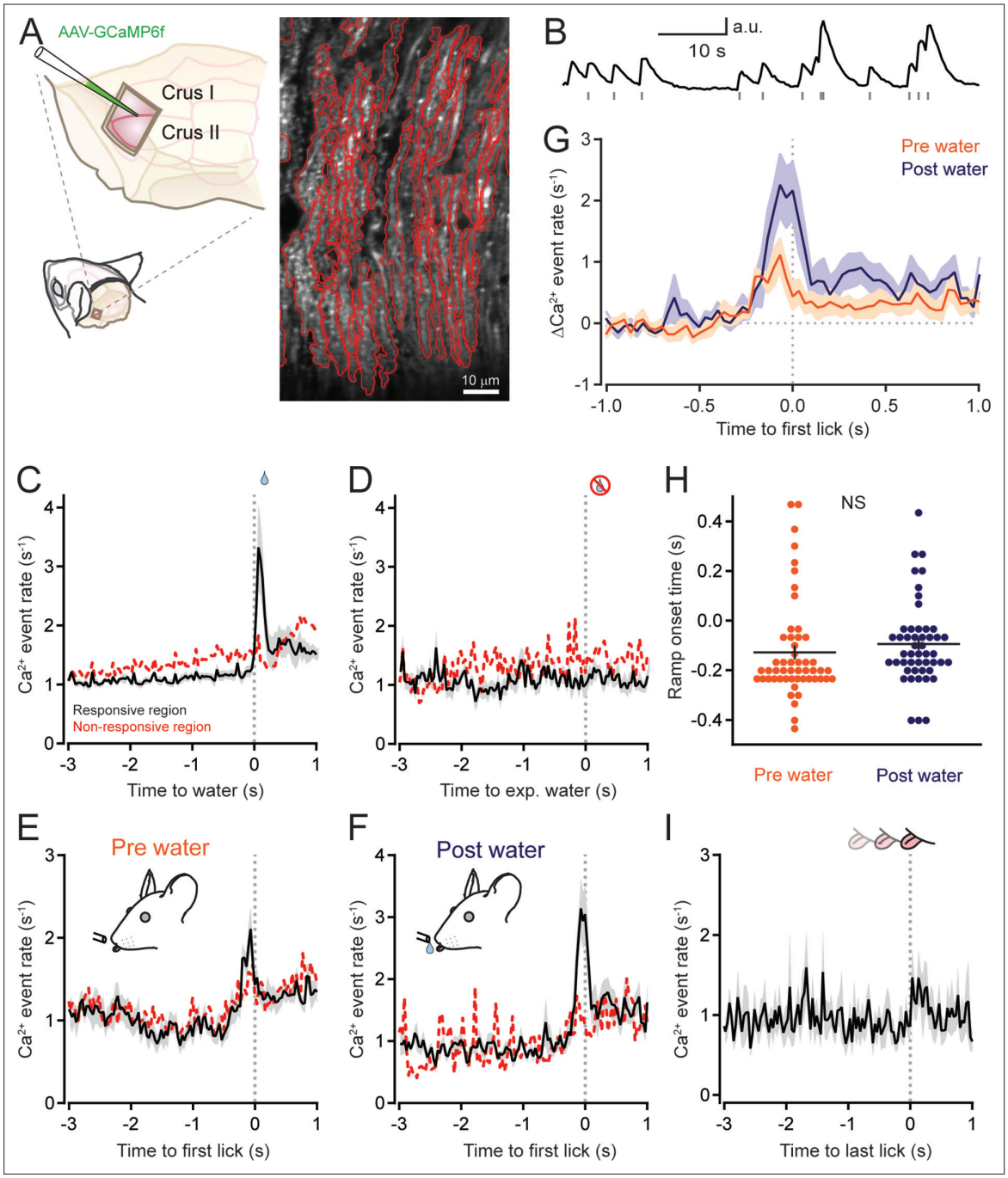


**Figure 6.** Climbing-fiber-evoked Purkinje cell (PC) activity increases at the initiation of discontinuous movement. (**A**) Left: AAV containing GCaMP6f under control of the *Pcp2* promoter was injected into Crus I and II to specifically transduce PCs; a cranial window provided optical access to the infected region. Right: a two-photon image with identified PC dendrites outlined in red. (**B**) Example fluorescence trace from a PC dendrite showing spontaneous calcium activity during quiescence. Individual climbing-fiber-evoked calcium events are indicated by gray tic marks. (**C**) Average climbing-fiber-evoked calcium event rates aligned to the time point of water delivery for water-rewarded trials. PC dendrites in some regions of Crus I and II showed clear increases in activity when mice elicited bouts of licking to water allocation (black line, $n$ = 377 PCs in 8 ROIs from 4 mice), whereas in other regions there was very little to no change in activity (dashed red line, $n$ = 239 PCs in 5 ROIs from 4 mice). (**D**) Same as panel C but for licking during unrewarded, water-omission trials. (**E**) Trial-averaged calcium event rates in PC dendrites aligned to the timing of the first lick in exploratory bouts initiated prior to water allocation (same data as panel C). (**F**) Same as panel E but aligned to the first lick for bouts of consummatory licking initiated after water allocation. (G) Top: overlay of the change in trial-averaged calcium event rates, relative to nonlicking baseline, for PCs in task-responsive regions of Crus I and II, aligned to the first lick of bouts initiated before or after water allocation (pre and post water, respectively). (**H**) Comparison of onset times for climbing-fiber-evoked calcium event ramping for individual PCs in trials where licking was initiated before (pre water; $n$ = 52 PCs) or after (post water;

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*n* = 49 PCs) water allocation (see Materials and methods). Black line shows the mean (not significant, NS; p = 0.36, Student's *t*-test). (**I**) Trial-averaged calcium event rate aligned to the timing of the last lick in trial bouts (*n* = 616 PCs, *n* = 12 sessions, 5 mice). See also ***Figure 6—source data 1***.

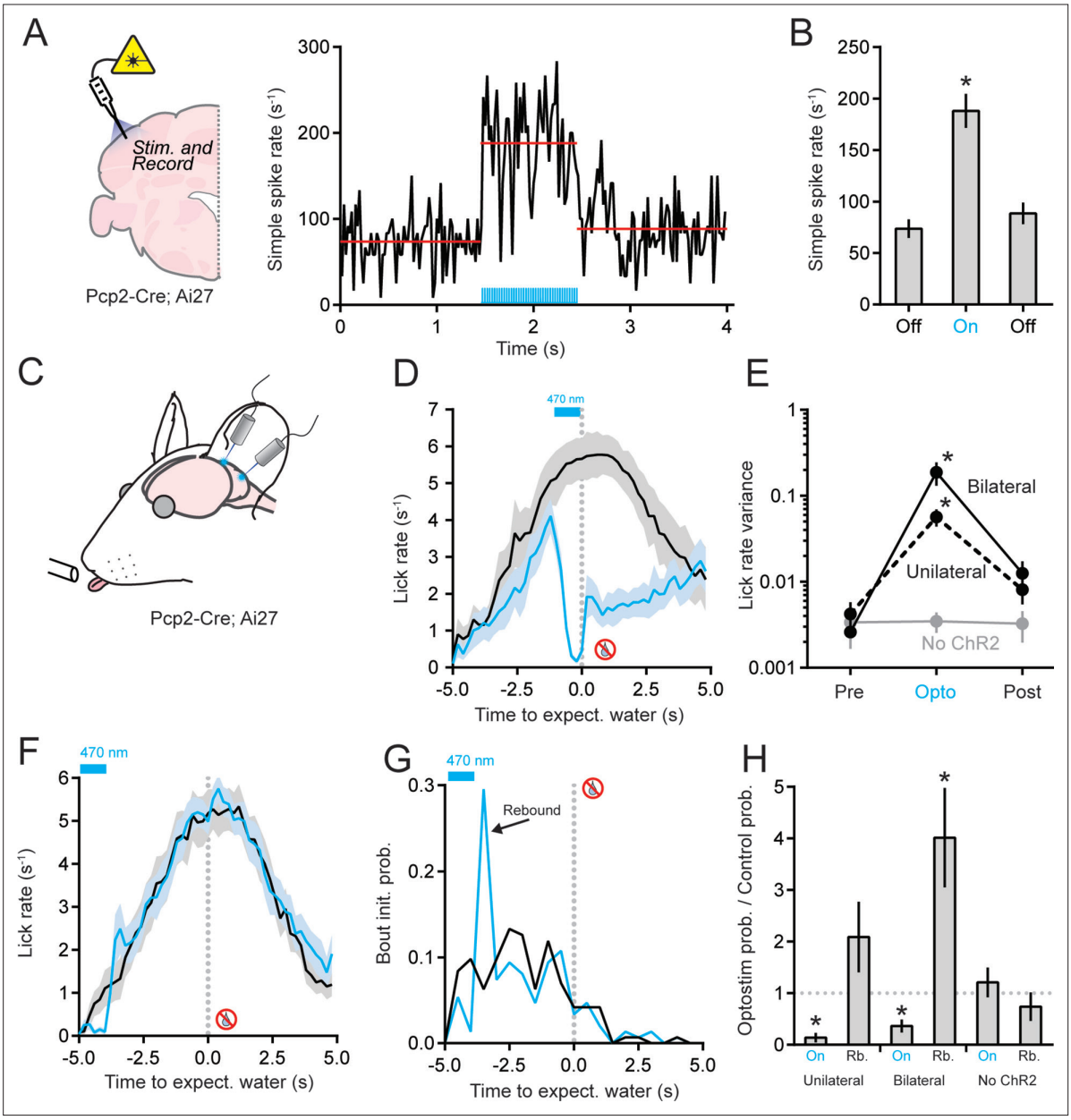


**Figure 7.** Optogenetic perturbation of Purkinje cell (PC) activity degrades the performance of discontinuous movements. (**A**) Left: extracellular electrophysiological measurements were obtained from ChR2-expressing PCs in response to photostimulation. Right: optogenetically induced simple spiking in a PC (light pulses indicated in blue). Red lines show the means for each epoch. (**B**) Summary plot of mean simple spike rate across PCs (*n* = 6) before, during, and after the optogenetic stimulus. Asterisk indicates significance ($p < 0.0001$, ANOVA with Tukey's post-test). (**C**) In mice with ChR2-expressing PCs, Crus II was bilaterally photostimulated during the interval task. (**D**) The effect of bilateral optogenetic PC activity perturbation on lick rate (blue) in unrewarded trials (*n* = 9 sessions, 3 mice). The photostimulus was timed to the period of peak licking, as referenced by interleaved control trials (black). Only well-isolated bouts were included in the analysis. (**E**) Summary of lick rate variability during optogenetic perturbation of PC activity. The *y*-axis is scaled logarithmically. Data include trials with bilateral photostimulation of Crus II (*n* = 9 sessions, 3 mice), trials with unilateral photostimulation of Crus I or II (*n* = 12 sessions, 4 mice), and trials in control mice where blue light was delivered bilaterally to the cerebellum but PCs did not express ChR2 (*n* = 6 sessions, 2 mice). Asterisks indicate significant differences during photostimulation trials ($p = 0.0228$ and 0.0199 for the unilateral and bilateral photostimulation conditions, respectively; ANOVA with Tukey's post-test). (**F**) Same as panel D but with the photostimulus timed to the period of earliest lick-bout initiations. Note the absence of licking during photostimulation and the large increase in licking immediately after photostimulation ended (*n* = 11 sessions, 3 mice). (**G**) Histogram of lick-bout initiation times for control and optogenetic stimulation trials (same data as panel F). A clear increase in licking probability is apparent after photostimulus ended. Only well-separated lick bouts were included (>2 s of prior nonlicking). (**H**) Summary of the effect of optogenetic PC activity perturbation on licking behavior during task performance. Asterisks indicate a significant reduction in licking during photostimulation (On: $p = 0.0079$ and $p = 0.0486$ for unilateral and bilateral stimuli, respectively; ANOVA with

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Tukey's correction for multiple comparisons) and a significant increase during the rebound period for bilateral stimulation (Rb: p = 0.0034, ANOVA with Tukey's correction for multiple comparisons). In the control condition, light stimuli were delivered to the cerebellum of non-ChR2-expressing animals (*n* = 12 sessions, 2 mice). See also ***Figure 7—source data 1***.

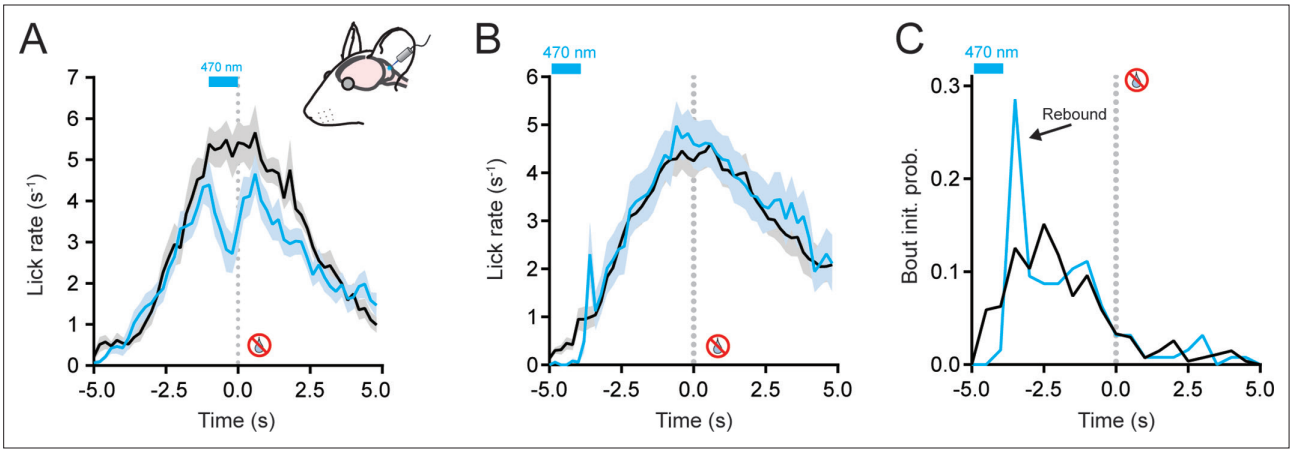


**Figure 7—figure supplement 1.** Unilateral optogenetic stimulation of Purkinje cells (PCs) during the task. (**A**) Unilateral optogenetic stimulation was delivered to ChR2-expressing PCs in left Crus I or II during the period of peak licking in water-omission trials. Comparison of average licking rates for stimulation trials (blue) as well as interleaved control trials (black) (*n* = 12 sessions, 4 mice). (**B**) As in panel A but with the photostimulus timed to the period of earliest lick-bout initiations (*n* = 20 sessions, 7 mice). (**C**) Histogram of the lick-bout initiation times. A clear rebound in lick probability is apparent immediately after the unilateral photostimulation ended.