Olivocerebellar control of movement symmetry

Graphical abstract

Authors

Vincenzo Romano, Peipei Zhai, Annabel van der Horst, ..., Xiaolu Wang, Joshua J. White, C.I. De Zeeuw

Correspondence

v.romano@erasmusmc.nl (V.R.),
c.dezeeuw@erasmusmc.nl (C.I.D.Z.)

In brief

Romano et al. show that bilateral movements are coordinated by cerebellar Purkinje cells. Depending on their anatomical location, Purkinje cells promote or reduce the symmetry of the left and right movements via complex spikes. The contextual inputs that modulate their simple activity regulate the effectiveness of complex spikes in a graded manner.

Highlights

- Complex spikes can promote or reduce the symmetry of left and right movements
- Cerebellar areas promoting symmetry are spatially distinct from those reducing it
- Simple spikes regulate impact of the complex spikes on symmetric movements
- Intracerebellar contralateral projections characterize areas promoting symmetry

Article

Olivocerebellar control of movement symmetry

Vincenzo Romano,^{1,3,4,*} Peipei Zhai,¹ Annabel van der Horst,¹ Roberta Mazza,¹ Thomas Jacobs,¹ Staf Bauer,¹ Xiaolu Wang,¹ Joshua J. White,¹ and C.I. De Zeeuw^{1,2,*}

¹Department of Neuroscience, Erasmus MC, Rotterdam, the Netherlands

²Netherlands Institute for Neuroscience, Royal Academy of Arts and Sciences, Amsterdam, the Netherlands

³Twitter: @in_romano

⁴Lead contact

*Correspondence: v.romano@erasmusmc.nl (V.R.), c.dezeeuw@erasmusmc.nl (C.I.D.Z.)

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SUMMARY

Coordination of bilateral movements is essential for a large variety of animal behaviors. The olivocerebellar system is critical for the control of movement, but its role in bilateral coordination has yet to be elucidated. Here, we examined whether Purkinje cells encode and influence synchronicity of left-right whisker movements. We found that complex spike activity is correlated with a prominent left-right symmetry of spontaneous whisker movements within parts, but not all, of Crus1 and Crus2. Optogenetic stimulation of climbing fibers in the areas with high and low correlations resulted in symmetric and asymmetric whisker movements, respectively. Moreover, when simple spike frequency prior to the complex spike was higher, the complex spike-related symmetric whisker protractions were larger. This finding alludes to a role for rebound activity in the cerebellar nuclei, which indeed turned out to be enhanced during symmetric protractions. Tracer injections suggest that regions associated with symmetric whisker movements are anatomically connected to the contralateral cerebellar hemisphere. Together, these data point toward the existence of modules on both sides of the cerebellar cortex that can differentially promote or reduce the symmetry of left and right movements in a context-dependent fashion.

INTRODUCTION

A wide range of simple and complex behaviors, varying from eye movements to spatial navigation, require coordinated movements of the left and right side of the body. For instance, locomotion or flying can only occur through a complex series of co-activations and alternations between sets of neurons and muscles on both sides of the body.¹ Hence, bilateral motor coordination is present and essential across the entire animal kingdom. Rodents, such as mice and rats, also exhibit a wide variety of coordinated bilateral motor behaviors, such as whisking, with flexible and adaptive levels of symmetry.^{2–5} Whisking is instrumental for nocturnal rodents to navigate in near total darkness;⁶ indeed, left and right whisker movements can occur at variable levels of symmetry and inform changes in head direction with respect to cues in the environment.² Moreover, given the rhythmicity of whisker movements, which are at least in part generated by a central pattern generator located in the intermediate reticular formation, this behavior may also be exploited to estimate the dynamic changes of head position over time.^{3,7–9} Whisker movements are controlled by retractor and protractor motor neurons in the facial nucleus,^{7,10,11} which receive input from a variety of structures in addition to the central pattern generator.^{8,12–14} Yet how the symmetry of movements on both sides of the body can be precisely coordinated is largely unknown.

A brain structure well known for its role in coordinating movements in general is the cerebellum.^{15,16} Ultimately, the cerebellar cortex integrates all of its inputs and directs all of its outputs via

its principal neuron, the Purkinje cell,¹⁷ typically considered to control the ipsilateral side of the body.^{18,19} The Purkinje cell fires two types of action potentials: simple spikes (SSs) and complex spikes (CSs).²⁰ SSs form the primary action potential of Purkinje cells and *in vivo* they occur regularly at 50–150 spikes per second, dependent on the microzone measured.^{21–23} Modulation of SS firing is correlated with many different types of sensorimotor behaviors and predominantly results from changes in activity of the mossy fiber-parallel fiber system, which is derived from an equally large variety of sources in the brainstem.^{24–29} CSs, on the other hand, occur less regularly and more slowly at a frequency of about 1 Hz.³⁰ Modulation of these all-or-none spikes results from activity in the climbing fibers,³¹ which are engaged during cerebellar learning and reward expectation^{32–34} and also during basic motor performance and reflexive reactions.^{4,35–39} Although all climbing fibers project unilaterally from the inferior olive to the Purkinje cells in the contralateral cerebellar cortex,⁴⁰ CSs on the left and right side can occur in synchrony,⁴¹ possibly due to bilateral inputs to the inferior olive and/or electrotonic coupling of dendrites passing across the midline.⁴²

Here, we set out to test the hypothesis that CS activity of Purkinje cells in the cerebellar cortex contributes to bilateral symmetry of whisker movements. Using electrophysiological recordings of Purkinje cells in the Crus1/2 region and optogenetic manipulation of their climbing fiber inputs while recording whisker behavior on both sides, we provide evidence that CS activity can encode and induce symmetric and asymmetric

Figure 1. Increased left-right coherence in CS-related whisking epochs

(A) Traces of representative recordings of whisker positions (top left) in the rostrocaudal plane and Purkinje cell (PC) recordings (middle left), as well as a schematic of the experimental set-up illustrating the head-fixed, restrained mouse with a high-speed camera allowing recording of the whiskers on both sides

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movements, dependent on the region involved. In addition, our data indicate that modulation of activity of neurons in the cerebellar nuclei induced by CS activity may play an instrumental role in facilitating symmetric movements via their contralateral projections. Moreover, we show that SS modulation preceding the CS modulation may strengthen its impact in the cerebellar nuclei neurons (CNNs) and thereby facilitate symmetric behavior of the left and right whiskers. Our data highlight that the role of the olivocerebellar system in coordination of movements extends beyond unilateral control, facilitating temporal integration of body dynamics on both sides.

RESULTS

Onset of symmetric whisking is associated with occurrence of CSs and vice versa

Spontaneous whisking is characterized by alternating periods of protraction and retraction in which the whiskers on the left and right side can either move together in an in-phase, symmetric fashion or they move asymmetrically with completely or partly out-of-phase behaviors. The level of symmetry in the behavior of the whiskers on the left and right side can suddenly change from, for example, an almost perfectly out-of-phase to an in-phase state with zero or almost no lag (Figures 1A and 1B; Video S1). The dominant frequencies of these asymmetric and symmetric behaviors cover the range from 3 to 30 Hz, and it is indeed within this range that one can observe prominent changes in coherence among the movements of the left and right whiskers (Figures 1C and 1D). To assess whether and to what extent symmetric whisker movements during spontaneous behavior are correlated with activity in the olivocerebellar system, we

recorded the CS activity from 127 single-unit Purkinje cells in lobules Crus1 and Crus2, which are known to encode whisker movements.⁴³ When we plotted the left-right magnitude-squared coherence just before and after the onset of whisking epochs, we observed that the coherence was significantly higher ($p < 0.001$) for epochs associated with CSs than those without (Figures 1C and 1D). To rule out the possibility that the higher coherence in whisking epochs with CSs was due to some epiphenomenon (such as longer whisking epochs having more probability of being associated with CSs), we compared the actual experimental data with data obtained by randomly shuffling the timing of the CS hundred times (Figures 1E and 1F; for details, see STAR Methods). In 17 out of the 127 cells, the mean difference in Z score during the first 500 ms, i.e., the period at which on average the CSs were observed, was higher than 2 (Figures 1F and 1G). Thus, for these cells the left-right coherence, and thereby symmetry, was higher than expected in whisking epochs with CSs, whereas it was less than expected in whisking epochs without CSs for the entire period of 500 ms (Figure 1E, left and medial panels). In contrast, only 8 Purkinje cells showed the opposite relation (Figures 1F and 1G). No significant differences occurred at the population level in the modulation of the SS activity during whisks with and without CSs (Figures 1H and 1I). Thus, the SS activity, even if it modulates relative to baseline firing rate during whisker movements, appears to be less clearly linked to whisker coherence than CS activity. This is in line with the finding that the modulation of SS encodes the whisker setpoint rather than the ongoing movement at each whisking cycle.⁴⁴

If CSs are indeed a reliable driver of left-right whisker synchronicity, we would expect to see a marked increase or decrease of

simultaneously (top right). Extracellular electrophysiological recordings are obtained using an electrode matrix placed unilaterally in the right hemisphere in lobules Crus1/ Crus2 of the cerebellar cortex. The average whisker positions of the left (contralateral, red) and right (ipsilateral, blue) sides transition from asynchronous to synchronous movements mode around the time at which two CSs (CSs, black dots) are fired in the PC (middle). The corresponding wavelet coherence plot (bottom) of left-right whisker movements shows the level of coherence increases in specific frequency ranges after the first CS and is further reestablished after the second CS. The black arrows indicate the difference of phase between the two signals (right, no phase difference; left, 180° difference of phase).

(B) Plot showing the distribution of phase differences of the whisker movements during the epochs of high coherence (i.e., magnitude-squared coherence or MSC > 0.9). Shaded lines indicate individual mice ($n = 8$) with the average in black.

(C) Whisking epochs associated with CSs (middle) were separated from those without (left). In the heatmaps, each line represents the average left-right whisker coherence (for frequencies from 3 to 30 Hz) for each recorded PC during whisker movement without (left) and with (middle) CSs. Zero represents the movement onset. The lines are sorted according to the differences obtained subtracting the two heatmaps (on the top the biggest left-right whisker coherence difference between whisks with and without CSs).

(D) The average level of left-right coherence (during the first 500 ms after the beginning of the whisker movements) was higher in whisking epochs with CSs ($p < 0.001$, paired t test). The coherence level of epochs with CSs minus those without CS was higher specifically in the frequency band of whisking (right) (wf, whisking frequencies = 3–30 Hz indicated in gray; lf, lower frequencies = 1–3 Hz in black, hf, higher frequencies = 30–300 Hz in white; one-way ANOVA, $p < 0.001$; post hoc test Bonferroni correction wf-lf and wf-hf, $p < 0.001$; lf-hf, $p > 0.05$).

(E) The observed level of coherence was compared between real and randomly shuffled CS timing (for details, see STAR Methods). These yielded the distributions of what could be expected by chance for each PC, and these distributions were used to calculate the Z score of the left-right whisking coherence.

(F) The histogram of the average Z score during the first 500 ms after the beginning of the whisker movement shows that in a minority of PCs (8 out of 127) the CSs are associated with less coherence than expected by chance for at least 500 ms ($Z < -2$). In 17 PCs, more coherence than expected by chance ($Z > 2$) was observed for the period of 500 ms in CS-related whisking epochs. Many other PCs crossed $Z > 2$ or $Z < -2$ within the first 500 ms after the beginning of the whisker movement.

(G) The resulting average CS activity of the 127 recorded PCs is shown in the period around the start of the whisker movements for the whisks without (left, magenta) and with (right, blue) CSs.

(H) The heatmap of the subtracted simple spike (SS) activity is shown for the individual PCs, sorted according to the difference in coherence of (E, right). Bigger differences in coherence do not correspond to bigger differences in SSs (as would be expected if SSs directly contributed to modulating the coherence). Though this panel is sorted according to the difference in coherence of (E, right), no more SSs are present in the top part of the panel (where more differences in coherence were present).

(I) The average SS activity relative to the whisking bouts with (blue) and without (magenta) CSs. The difference in SS modulation (from 0 to 500 ms after the movement onset) was not significant at population level ($p = 0.149$, Wilcoxon matched-paired signed test). The shaded areas represent the inter-quartile ranges.

Figure 2. Left-right cross-correlation increases around the occurrence of a CS

(A) Examples of left (red) and right (blue) whisker traces associated with CSs (black dashed lines). Yellow shadows indicate 150 ms before and 300 ms after the CS occurrence.

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symmetry just after the occurrence of a CS. To test this, we quantified the level of left-right symmetry around the time of CSs using cross-correlation analysis. The cross-correlation of left and right whisker movements was calculated using a sliding window (see STAR Methods and Figure S1 for details), and the result was compared with randomly shuffled CS timing. The level of cross-correlation was increased around the time of a CS occurrence in 34 out of 127 cells (Z score > 2 in the 125 ms around the CSs), indicative of synchronous, symmetric whisker movements (Figures 2A–2E). Importantly, for the cells with the highest cross-correlation Z scores (top part of the middle panel of Figure 2E), the CSs occurred at a transition between epochs of low and high left-right symmetry. The left-right symmetry was bimodally distributed; in 21 out of 127 cells, a CS was associated with a decrease in symmetry (Z score < -2), highlighting the induction of asymmetric, out-of-phase, movements (Figures 2C, 2D, 2E right, and 2F). In general, when considering all 127 cells, the timing of the maximal change of Z score, relative to the CS time, was approximately normally distributed with the highest symmetry often occurring after the CS (Figure 2G). This became particularly evident when considering only the 55 cells showing a significant correlation (Z score > 2 or Z score < -2) (Figure 2G, inset).

Next, we wondered if the cells with positive and negative Z scores of their CS responses were intermingled or differentially distributed within Crus1 and Crus2. The olivocerebellar system, including Crus1 and Crus2, is known to be organized in micro-modules with their Purkinje cells and climbing fibers contributing to specific functions.^{21,45,46} By plotting the maximal Z scores of symmetry of each cell on the approximate location where the Purkinje cell was recorded^{4,47} (see STAR Methods for details), it was possible to distinguish areas populated with cells with positive Z scores (such as medial Crus1 and lateral Crus2) from other areas where negative Z scores tended to be concentrated (medial Crus2) (Figure 2H). The Z scores of CS responses in lateral Crus1 showed an intermediate level. These results suggest that CS activity in medial Crus1 and lateral Crus2 is associated with more left-right whisker symmetry, whereas CS activity in medial Crus2 is associated with asymmetric movements.

CS activity and kinematics of whisker protractions

Several independent lines of evidence have shown that transient increases of SS frequency can contribute to whisker protraction.^{4,44,48,49} Our results are indicative of a role for CSs in bilateral whisker synchronicity, i.e., symmetry. If CSs themselves are involved in whisker movement, we can expect that the kinematic profile of whisker protractions associated with CSs is different from that of randomly selected whisker protractions. Therefore,

we again shuffled the CS timing 100 times and randomly selected CS-related whisker protractions. These were used to calculate the Z score of the whisker angle and angular velocity of the actual CS-related whisker protractions (Figures 3A–3D). This method allows us to correct for the bias that whisker protractions with longer duration have a higher chance of being associated with a CS. The length of time of individual whisker protractions was normalized to be from 0 to 1 (see STAR Methods and Figure S2 for details). In a subset of Purkinje cell recordings, we found that CSs were associated with larger and faster whisker protractions, compared with those selected after shuffling the CS times (Figure 3). However, this was not apparent when we averaged all recordings (Figures 3E–3H). The phase of maximal Z score was heterogeneous for the different Purkinje cells (Figures 3I and 3J). Accordingly, the Z scores of the velocity profile of whisking were also different from the randomized data for the different subsets of cells (35 out of 127 cells for the ipsilateral side compared with 27 out of 127 of the contralateral side) (Figures 3K and 3L). Thus, CSs in a subset of Purkinje cells may be capable of influencing whisker movements in differential ways.

SS rate before a CS predicts the magnitude of whisker protractions

Because SSs do modulate during CS-related whisker protractions (Figure S3), we next examined their potential role in regulating the impact of a CS on whisker protraction. It has been theorized that Purkinje cell activity may affect fast reaction movements in part via disinhibition of the CNNs.^{15,50} More specifically, a pause in SS activity caused by a CS may induce rebound activity of the CNNs,^{15,32,51} which, in turn, may excite premotor neurons located in the brainstem.⁴⁹ The level of SS activity prior to a CS has been shown to influence CS spikelet count, indicating a potential impact on the strength of the signal.⁵² Here, we hypothesize that the contextual state provided by the mossy fiber/parallel fiber input system, which modulates SS activity,⁵³ may increase the intensity of disinhibition of the CNNs and thereby the activity of downstream premotor whisker neurons.¹⁵ If our hypothesis is correct, whisker protractions associated with CSs preceded by a high SS rate will be larger than those in which the preceding SS rate is lower. Therefore, we calculated the instantaneous SS rate (ISSR) by taking the reciprocal of each inter-spike interval and then convolved those values to obtain a time series in which the ISSR is represented as a continuous vector with 1 ms time resolution (see STAR Methods and Figure S4 for details). Using the ISSR, we sorted the CS-related whisker protraction epochs into three groups depending on the ISSR at the time that the CS occurred. We considered the ISSR during the 5 ms prior to the CS (STAR Methods). The three groups

(B) Enlargement of (A). Epochs of a high correlation between left and right whiskers can be observed both before and after CSs.

(C–E) Same as (A) and (B) (C and D) but for a PC for which the CSs are associated with asymmetric whisker movements (E, left). The Z score of the observed cross-correlation around the CS time is shown for all 127 PCs. The data are sorted according to the mean Z score in the first 125 ms after the CS. In this time window, 34 out of 127 cells had a $Z > 2$ (E, middle), while 21 out of 127 had $Z < -2$ (E, right).

(F) The histogram of the maximum Z score of all 127 cells.

(G) Latencies corresponding to a maximum change of Z scores between two consecutive bins (calculated as a differential of the Z score). Note that the maximal changes of Z scores are observed around the time at which the CSs occurred. When considering only the 55 PCs with $Z < -2$ or $Z > 2$ the change in Z score occurs after the CS.

(H) Plotting the maximal Z scores on the approximate location where the PCs were recorded (indicated by the white dots on the schematic map), it is possible to distinguish an area with prevalent negative Z scores (in medial Crus2) from two areas with prevalent positive Z scores (Crus1 and lateral Crus2). See also Figure S1.

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were defined by using the mean and the standard deviation (SD) of all the ISSR values: more than 0.5 SD below the mean, low, within 0.5 SD above or below the mean, middle, and greater than 0.5 SD above the mean, high. Most (82 out of 127) Purkinje cell recordings contained all three groups during whisker movements and could be used for this analysis. In line with our prediction, CS-related whisker protractions were larger in amplitude and faster in velocity for both ipsilateral and contralateral whisker movements when the ISSR was higher around the CS timing (Figure 4). Notably, the preceding ISSR had the largest impact on the amplitude of the ipsilateral whisker movement (Figures 4E–4H). Using the ISSR classification, only the CS-related whisker protractions with a low ISSR were not significantly different from the corresponding randomized data (at a population level, the low pre-CS ISSR Z scores did not exceed ± 2 ; Figures 4A, 4E, 4I, and 4M). Middle and high pre-CS ISSRs were associated with average Z scores that abundantly exceeded ± 2 . This indicates that those CS-related whisker protractions were larger and faster compared with the corresponding randomized data. These results cannot be attributed to a direct impact of the ISSR itself because the Z score was calculated by comparing whisker protraction with high ISSR and CS to whisker protraction with the high ISSR but without a CS (i.e., by randomly shuffling the CS timing). Thus, the relationship between the preceding ISSR and the magnitude of whisker protraction suggests a graded, but real, impact of the CS on movement.

Optogenetically induced CSs drive either symmetric or asymmetric whisker movement

To directly test the impact of CSs on movement, we optogenetically activated climbing fibers and evaluated the resulting whisker movements. We rendered the climbing fibers sensitive to light by injecting an AAV to drive expression of the red-light drivable channel-rhodopsin, Chrimson,⁵⁴ into the inferior olive. After 6 weeks of incubation, we performed a craniotomy over the cerebellum and optically stimulated the Crus1/2 regions of the cerebellar cortex, while simultaneously recording Purkinje cells and whisker movements. Optogenetic stimulation induced CSs within 3–6 ms in nearly all trials when the recorded Purkinje cell was close to the optic fiber (Figures 5A–5D and S5). This always resulted in a SS pause lasting at least 20 ms (Figure 5C). When we evaluated the impact of climbing fiber activation in different areas of Crus1 and Crus2, we found reliable movement responses to our

stimulation with different features depending on the stimulated regions. Notably, the fact that the light stimulation of the cerebellar cortex induces a response peak that starts before the peak induced by olivary neuron stimulation indicates that CSs are not generated by antidromic activation of olivary neurons (Figure S5B). Symmetric whisker movements on the left and right were predominantly induced when stimulating medial and lateral Crus1, as well as lateral Crus2 (Figures 5G and S5D). Instead, asynchronous, bilateral whisker movements were induced when stimulating medial Crus2 (Figures 5F–5H, S5C, and S5D). In addition, stimulation of the rostrolateral part of lateral Crus1 could also elicit ipsilateral movements only. These results demonstrate that CSs do not simply affect whisker movements but can trigger specific patterns of unilateral or bilateral whisker movements depending on the location in the cerebellar hemisphere. The finding that bilateral asynchronous whisker movements were induced in the same area where spontaneous CSs were associated with less left-right symmetry (Figure 2H) suggests that the CSs in that area promote asynchronous whisker movement during natural behavior. Conversely, CSs in the surrounding areas are likely to promote increased left-right synchrony, i.e., symmetry.

Bidirectional modulation of cerebellar nuclei activity encodes CSs and synchronous whisker movements

If CSs regulate the symmetry of bilateral whisker movements partly via the activity in CNNs,¹⁵ the level of symmetry should correlate with the magnitude of the CNN activity modulation. To test this hypothesis, we calculated the coherence between left and right whisker movements over time and correlated it with the firing rate of CNNs (Figure 6). We built a matrix of correlation by calculating Pearson's correlation coefficients at 10 ms intervals (from –200 to +400 ms relative to whisker movement onset) of the individual epochs of movements^{32–34} (see STAR Methods for details). When the Pearson's correlation coefficients of a CNN after movement onset crossed the threshold of two SDs above the mean before movement onset (from –200 to –100 ms), we considered that CNN significantly correlating with bilateral whisker coherence. In 23 out of the 73 CNNs, we found a significant positive correlation (Figures 6C–6F). Thus, in these 23 CNNs, more spike activity was associated with symmetric bilateral whisker movements. In 24 out of 73 CNNs, we found a significant negative correlation instead; a reduction of their activity was associated with symmetric bilateral whisker

Figure 3. Larger whisker protractions related to CSs in a subset of PCs

(A) Left: example of left side (contralateral to recording side) showing the average CS-related whisker protractions (magenta line) compared with those following randomly shuffling the CS timing 100 times (the gray shadow indicates the interval of confidence between the 1% and 99% of the randomized data). Both the whisker position during the protraction and the corresponding average velocity profile (right) for this example PC are larger than what was expected by chance. (B) Similarly, the protraction of the right-side whiskers is enhanced in protractions related to CSs. (C and D) This can be observed by calculating the Z score of the protractions, which is often higher than 2. The duration of the protraction is normalized to 1 (see also Figure S2). (E–H) The result observed in this specific PC did not emerge at the population level. (I and J) The Z score of the individual 127 PCs is shown as a heatmap for the position and the corresponding velocity of left (I) and right (J) CS-related whisker protraction. The data are sorted according to the phase of the maximal Z score of the velocity profile of the right whisker side (J, right). (K and L) By plotting the maximum and minimum Z scores of position and velocity profiles, it is possible to see that, at individual cell level, several CS-related whisker protractions have a $Z > 2$ or $Z < -2$. This is shown for the left (K) and the right (L) side-whiskers, where the data are sorted according to the maximal Z score of the velocity profiles. At the bottom, the pie charts indicate the fraction of cells for which the CSs are associated with bigger and faster whisker protractions (red) or smaller and slower (blue). Note that the most extreme Z score values are observed for faster right whisker protraction. This suggests that the occurrence of CS is mostly, but not exclusively, associated with faster ipsilateral whisker protractions. See also Figures S2 and S3.

Figure 4. SS rate before the CS predicts the magnitude of whisker protractions

Based on the instantaneous SS rate (ISSR) at the moment the CS occurred, we divided the CS-related whisker protractions into 3 groups: low (blue), middle (green), and high (red) ISSR before the CS (see STAR Methods for details).

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movements. Thus, when bilateral whisker movements were more symmetrical, some CNNs increased and some others decreased their spike activity. We also observed a bidirectional modulation when CNN spike activity was aligned to the CS timing of simultaneously recorded PCs (Figure S6). Taken together, these results suggest that the impact of CSs on the symmetry of bilateral whisker movements could be mediated by bidirectional modulation of CNNs.

Regions related to whisker symmetry share anatomical connections with the contralateral cerebellum

Given the relationship between cerebellar activity and bilateral movement of the whiskers, we explored the potential anatomical correlates. Indeed, differences in evoking left-right symmetric movements should require different anatomical connectivity between the region in which CSs are associated with asynchronous movements (i.e., medial Crus2) and the regions associated with synchronous bilateral movements (i.e., medial Crus1 and lateral Crus2, and to a lesser extent also lateral Crus1). To investigate the differences in the connectivity of Purkinje cells of these different subregions, the anterograde trans-synaptic tracer AAV-CAGsm-myc⁵⁵ was injected in discrete regions of Crus1 and Crus2 in 6 mice. After a survival period of 10 weeks, the animals were sacrificed, and their brains were processed for histology. Depending on the location of the injection site, GFP labeled fibers were found in the cerebellar nuclei on both the ipsilateral and contralateral sides, in the ipsilateral and contralateral cerebellar hemispheres, as well as in brainstem nuclei on both sides (Figures 7A–7C and S7). Mossy fiber terminals were apparent throughout the cerebellar cortex contralateral to the injection (Figure 7) except for injections into medial Crus2 (Figures 7D–7F). In addition, whereas GFP labeled fibers were always found in the ipsilateral cerebellar nuclei, labeling in the contralateral cerebellar nuclei was found following injections in mediolateral Crus1, as well as lateral Crus1 and Crus2, but not following those in medial Crus2, i.e., the area that showed asymmetric movements following optogenetic stimulation of their climbing fibers. Contralateral projections via the cerebellar nuclei likely originate from glutamatergic neurons as injections directly into the cerebellar nuclei only led to contralateral labeling in Vglut2-cre, but not Gad2-cre mice (Figure S7). The former allows for viral targeting specifically to glutamatergic, whereas the latter only targets GABAergic neurons. Thus, the Purkinje cells of both medial Crus1 and lateral Crus1/Crus2 are anatomically connected via mossy fibers to the input stage of

the opposite cerebellar hemisphere via the cerebellar nuclei. These results suggest that only the modules in Crus1 and Crus2 that appear to play a role in coordinating symmetric movements of the left and right whiskers are connected to their counterpart modules in the contralateral cerebellar cortex.

DISCUSSION

Whereas crude control of left-right movements can be readily established in brainstem, spinal cord, or cerebral cortex, the mechanisms underlying fine-regulation of symmetric movements have remained largely enigmatic. Here, we explored the role of cerebellum in bilateral coordination of movements by investigating the impact of Purkinje cell activity on left-right synchronicity of whisking. We found that CSs in Purkinje cells of lobules Crus1 and Crus2 can induce, in addition to unilateral whisker movements on the ipsilateral side, bilateral symmetric and asymmetric movements, depending on the precise location within the cerebellar hemisphere. The effectiveness of this climbing fiber control of bilateral symmetry appears to depend on the context provided by the status of the mossy fiber/parallel fiber system in that increased SS activity preceding the CS response results in a stronger effect. Moreover, enhanced synchronous whisker movements on the left and right side come with increased activity in a subset of CNNs, possibly following the imprinting of the CSs. Interestingly, only the Crus1 and Crus2 areas that were able to induce synchronous symmetric whisker movements on the left and right side were found to share anatomical connections with the contralateral cerebellar hemisphere. Our results indicate that the olivocerebellar system plays an instrumental role in coordinating symmetric movements.

Neuro-anatomical substrates for bilateral control

For medial Crus1 and lateral Crus2, all data were consistent in that the modulation of CS activity during bilateral synchronous whisker movements, the impact of stimulation of the climbing fibers on bilateral whisker movements, and the *trans*-neuronal Purkinje cell projections to the contralateral cerebellar hemisphere all perfectly aligned, converging into a parsimonious role in coordinating symmetric movements. Likewise, the datasets on medial Crus2 were consistent in that neither the CS recordings, nor the optogenetic stimulation, nor trans-synaptic tracing experiments suggested a role in control of synchronization of bilateral movements for this area. However, the data on lateral Crus1 were

(A) The average whisker protraction (left whisker side) for each of the three groups is shown together with what was obtained by randomly shuffling the CS time 100 times (gray shaded areas indicate the interval of confidence between the 1st and 99th percentile of the randomized data). The higher the SSR before the CS, the bigger the amplitude of the CS-related whisker protractions.

(B) On the left, the average whisker protractions are overlaid for comparison. On the right, the cumulative histogram of the amplitude of the protractions relative to each recorded PC is shown. Only PCs for which all three levels of ISSRs could be detected were included in this analysis.

(C) A similar trend could also be observed when calculating the Z score of observed data relative to the distributions obtained with randomly shuffled data. The heatmaps represent the Z score of the protraction profiles relative to each PC.

(D) The average Z score of the entire population is shown on the left and the maximal Z scores of the overall protraction amplitude relative to each individual cell is shown, as a cumulative histogram, on the right.

(E–H) The relationship between the level of ISSs before the CS and the magnitude of whisker protraction is even stronger on the right whisker side, which is ipsilateral to the recorded PC.

(I–P) The same result can be observed by repeating the same analysis on the velocity profile rather than position. Note that for both left (I–L) and right whiskers (M–P), when the preceding ISSR was low, the CS-related whisker protractions presented a slower velocity profile, which was not different from what was expected by chance. Faster velocity profiles were instead observed for higher ISSRs preceding the whisker protraction-related CSs (L and P).

See also Figure S4.

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somewhat more ambiguous; whereas the optogenetic stimulation and tracing experiments indicated that this area is also predominantly, but not exclusively, involved in coordination of bilateral symmetric movements, the Z scores of the CS responses showed intermediate levels. Possibly, adjacent microzones in lateral Crus1 are narrower than in the other subregions studied,^{46,56} hampering precise reproduction of the outcomes of the recording and stimulation experiments. Therefore, our stimulation methods are not specifically capable of testing the relationship between the Zebrin-based modular map^{23,57} and synchronous behavior. However, our stimulation maps are suggestive of an anatomically organized response profile of Purkinje cells to specific whisking behavior.

It was interesting to observe that Purkinje cells that were able to induce symmetric movements via optogenetic activation of their climbing fiber input were found to be anatomically connected with contralateral cerebellar cortical areas. In other words, it appears that the olivocerebellar modules involved in coordination of symmetric movements on the left and right side (i.e., in particular the left and right, medial Crus1 and lateral Crus2) are reciprocally connected with each other, whereas those involved in asymmetric movements (i.e., in particular the left and right, medial Crus2) do not show this pattern. This configuration suggests that Purkinje cells in the cerebellar cortex play an important role in fine-tuning symmetric movements. Questions remain regarding how Purkinje cells coordinate left-right synchronization under physiological circumstances. Given the temporal delays generated by the commissural connections between the left and right cerebellar cortices and given the fact that all climbing fiber projections are purely unilateral in that they are all contralateral,⁴⁰ one may wonder how the left and right whiskers can move symmetrically with often zero phase lag. In addition to some bilateral inputs to particular olivary subnuclei, as well as dendritic electronic coupling in the T-area between the left and right inferior olive,⁴² the main answer to this question can probably be found in the temporal prediction mechanisms of the olivocerebellar circuitry in general.⁵⁸ Guided by the timing of their climbing fiber inputs, cerebellar Purkinje cells can enhance or suppress specific signals of the mossy fiber/parallel fiber pathway and adjust the timing of their spiking with millisecond precision.^{59,60} Thus, as we show here that Purkinje cells on the left and right sides are reciprocally connected by targeting the

CNNs that provide a nucleo-cortical projection via the mossy fibers,^{61–64} one can speculate that the context signals mediated by the mossy fibers can be accelerated through the adaptive plasticity mechanisms that depend on the timing of the climbing fiber activity and thereby minimize potential phase lags when necessary.^{40,60} Upstream, this mechanism of time-shaping in the molecular layer of the cerebellar cortex may be facilitated by the fact that many of the mossy fiber projections from extracerebellar sources to the Crus regions are also bilateral.^{65–68} Moreover, downstream, similar arguments may hold in that the outputs of the cerebellar nuclei that are innervated by the Crus regions also comprise bilateral components.⁶⁹ Indeed, in human the dentate nucleus projects both via a decussating and a non-decussating pathway to the nucleus ruber and thalamus,^{70,71} whereas in rodents both ipsilateral and contralateral projections have been shown from the various cerebellar nuclei to the brainstem,^{72,73} including the areas that affect whisker movements.^{8,13,72,74,75} However, it should be noted that in most of these cases the downstream connections are not perfectly symmetric but rather project to only partially overlapping areas within the upper or lower brainstem on the left and right side. Thus, we propose that intracerebellar communication in which a copy of the cerebellar cortex output can reach the contralateral cerebellar hemisphere and be fine-tuned in time by the climbing fiber system, forms one of the main anatomical substrates for synchronicity of symmetric movements on the left and right side.

Ethology of symmetric and asymmetric whisker movements

Mice are nocturnal and mostly active in the dark, often using their whiskers to explore the environment similarly to how humans use their vision during daylight.⁷⁶ Rodents sweep their whiskers in concert with other autonomic or voluntary rhythmic behaviors, such as respiration, locomotion, sniffing, and grooming.^{9,47,77–79} The two sets of whiskers can move synchronously or asynchronously depending on the behavioral context. For instance, asymmetric whisker movements can predict a turning of the head.² This internal preparation could preserve the stability of the whisker position in space during locomotion in the dark similar to the preparatory eye movements in diurnal mammals that stabilize gaze during head movements.^{80,81} Indeed, just like Purkinje cells of the cerebellar flocculus that can accelerate eye movement

Figure 5. Optogenetic stimulation of climbing fibers triggers symmetric or asymmetric whisker movements depending on the cerebellar region

(A and B) AAV-syn-ChR2-mCherry virus was injected into the left inferior olive, and after 6 weeks of incubation, CSs could be reliably induced by applying orange light on the contralateral cerebellar cortex.
(C) Left: raster plot and histogram of 25 light stimuli show that CSs could be induced at nearly every climbing fiber stimulation. Right: optogenetic activation of climbing fibers also led to a pause in SSs following the CS.
(D) The convolved peri-stimulus time histogram (PSTH) is shown for 9 PCs recorded at a variable distance from the optic fiber (300–2,000 μ m). CS PSTHs are shown on the top and the corresponding SS PSTHs below.
(E) An example of a whisker movement induced by three consecutive light stimulation (in lateral Crus2) is shown. The left (red) and right (blue) side of the whisker move with a high level of synchrony.
(F) The pattern of movements in the opposite direction (asymmetric) was instead induced by stimulating climbing fibers located more medially.
(G) The average whisker responses (for one example mouse) are plotted in the approximate location where the light stimulation was applied. Note that the different patterns of movements were induced by stimulating different locations. Shaded areas indicate the SEM.
(H) Synchronicity between left and right whisker movements was calculated using the cross-correlation with a sliding window (as in Figure 2C; see STAR Methods for details) and the resulting Z score is shown. Each heatmap represents the level of left-right whisker correlation resulting from the light stimulation in that approximate location. Each horizontal line represents the Z score of left-right whisker cross-correlation for individual mice (n = 5) around the time of the stimulation (from –200 to +400 ms). As for the example in (G), less synchronous bilateral whisker movements were observed when the stimulation was delivered in medial Crus2.

See also Figure S5.

Figure 6. Bidirectional cerebellar nuclei neuron (CNN) modulation during symmetric whisker movements

(A) Raw trace of an exemplary PC (black) simultaneously recorded with a CNN (blue) and sorted spike times (below). The increased CNN spike activity is associated with a symmetric movement.

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signals,^{82,83} Purkinje cells of medial Crus2 might contribute to the preparatory whisker movements that are engaged just before active head rotation. Likewise, during different voluntary behaviors, such as sniffing, Purkinje cells of medial Crus1 may coordinate symmetric whisking with the more autonomous respiratory cycle.⁴⁷ Or Purkinje cells of lateral Crus1 and Crus2 may align the level of symmetry of whisker movements with the left-right limb coordination during alternating left-right movements like locomotion.^{16,37,84} Thus, on the one hand Purkinje cells in the Crus1 and Crus2 areas appear to be well designed to engage preparatory activities of whisker movements on both sides of the body, and at the same time, they may adjust the kinematics of this type of behavior to those of others depending on the environmental demands.

Differential behavioral encoding by CSs

Historically, the olivocerebellar system is mainly associated with motor coordination based on the effect of cerebellar damage in human patients, as well as the results of laboratory studies in animals.^{15,37,48,85–90} However, recently evidence has been accumulating for non-motor functions of the cerebellum.^{91–96} Several studies have suggested that the cerebellum also contributes to planning and decision making, as well as reward expectation.^{33,34,92,96} Accordingly, when deciphering what CSs encode, one can find evidence not only for motor^{32,39,60,97–103} but also non-motor functions.^{33,34,95,96,104} For example, changes in CS activity have not only been associated with induction of reflexive movements and adaptation of movements^{4,26,32,35,105} but also with encoding of preparatory signals, as well as anticipation and evaluation of reward.^{33,34,95,96,104} Thus, given that we show here that climbing fiber-induced CS activity can evoke the initiation and maintenance of specific patterns of bilateral movements, it will be interesting to find out to what extent it can also contribute to bilateral coordination of non-motor signals, such as connecting sensory discrimination in one of the cerebral cortices with decision making in the cortex on the other side. Likewise, it will be interesting to find out whether CS patterns can contribute to coordinating motor with non-motor activity on different sides of the brain.⁷⁵

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2021.12.020>.

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AUTHOR CONTRIBUTIONS

V.R., P.Z., and A.v.d.H. designed and performed experiments. V.R., P.Z., A.v.d.H., and J.J.W. analyzed the data and wrote and revised the article. R.M., T.J., and S.B. analyzed the data and contributed to data visualization. X.W. contributed to performance of the experiments. C.I.D.Z. supervised the project, provided financial support, and wrote the paper. V.R., P.Z., and A.v.d.H. contributed equally to this work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure sex balance in the selection of non-human subjects.

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(B) The matrix of correlation between whisker left-right coherence (magnitude-squared coherence or MSC) and spike rate of the same exemplary CNN of (A). The color of the heatmap represents the Pearson's correlation coefficient around the onset of the whisker movements (see also STAR Methods for details). The red-yellow color indicates that in an epoch of whisker movements where the coherence (between left and right whisker movements) is high that CNNs fired more spikes and vice versa. When the Pearson's correlation coefficient during the movement crossed two standard deviations of its value prior to the movements (from −200 to −100 ms to the movement onset), the CNNs were considered significantly correlating.

(C) The matrix of 23 out of 73 CNNs correlated with more coherence. Shaded areas indicate the SEM.

(D) The raw trace of another exemplary PC (black) simultaneously recorded with another CNN that suppressed its firing rate during symmetric bilateral whisker movements.

(E) Same as (B) but for the CNNs shown in (D). Here, the blue-white color indicates that CNNs fired less spikes in an epoch of bilateral whisker movements where the coherence is high.

(F) The matrix of correlation of 24 out of 73 CNNs for which less spikes correlated with more coherence. Shaded areas indicate the SEM.

See also [Figure S6](#).

Figure 7. PCs of Crus1 and lateral Crus2 are more trans-synaptically connected to the contralateral cerebellum than PCs of medial Crus2

(A) Schematic of the experimental procedure of injection of AAV-CAGsm-myc in Crus1.

(B and C) Injection in lateral Crus1 results in labeling in the contralateral cerebellar cortex (C, top) and DCN (C, bottom).

(D) Schematic of the experimental procedure of injection of AAV-CAGsm-myc in medial Crus2.

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(E and F) Injection in medial Crus2 did not result in any staining in the contralateral cerebellum.

(G) Schematic of the experimental procedure of injection of AAV-CAGsm-myc in mediolateral Crus1/2.

(H and I) Injection in mediolateral Crus1/2 across the horizontal fissure also results in labeling in the contralateral cerebellar cortex but to a lesser extent.

(J–O) Injection in medial Crus1 also results in labeling, such as mossy fiber terminals in the contralateral cerebellar cortex (M–O). Dots represent locations where terminals were found. PM, paramedian lobule. |

See also [Figure S7](#).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti-myc primary antibody (1:10000)	Abcam	ab9132; RRID: AB_307033
Alexa fluor 488 donkey anti-guinea pig secondary antibody (1:400)	Jackson	706-545-148; RRID: AB_2340472
Bacterial and virus strains		
AAV5-hSyn-Cre-eGFP	Addgene	RRID: Addgene_105540
AAV1-CAG- GFPsm-myc	Addgene	RRID: Addgene_98926
AAV9-hSyn-FLEX-tdTomato	Addgene	RRID: Addgene_59171
Chemicals, peptides, and recombinant proteins		
Paraformaldehyde	Merck	1.040005.1000
Thionine	Sigma	T-3387
Gelatin	J.T.Baker	2124-01
Bupivacaine	Actavis	RVG 20949
Lidocaine	Braun	RVG 07831
Buprenorphine	Reckitt Benckiser Pharmaceuticals	RVG 08725
Rimadyl	Pfizer	CAS 53716-49-7
Isoflurane	Karizoo	10060000013
Super-Bond C&B	Sun medical	075956
Experimental models: Organisms/strains		
C57BL6/J mice	Charles Rivers	IMSR_JAX:000664
Gad2-ires-Cre	Own breeding	No. 010802
VGlut2-ires-Cre	Own breeding	No. 016963
Ai27D	Own breeding	No. 012567
Software and algorithms		
MATLAB	Mathworks	N/A
LabVIEW	National Instruments	N/A
BWTT Toolbox (for whisker tracking)	http://bwtt.sourceforge.net	150622-BWTT-v1.0.1-R17.zip
Custom whisker tracking code	https://github.com/MRIO/BWTT_PP	N/A
SpikeTrain	Neurasmus	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled with the Lead Contact, Vincenzo Romano (v.romano@erasmusmc.nl).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data are available from the Lead Contact upon request. The custom code complementing BWTT whisker tracking can be obtained via https://github.com/elifesciences-publications/BWTT_PP. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

All animal experiments were approved by the institutional animal welfare committee of Erasmus MC in accordance with the Central Authority for Scientific Procedures on Animals guidelines. Wild-type C57BL/6J (No. 000664), Gad2-ires-Cre (No. 010802), VGLUT2-ires-Cre (No. 016963), and transgenic Ai27D (No. 012567) mice were obtained from the Jackson Laboratory. All mice in this study were 6–34 weeks old and were housed individually in a 12-hours light-dark cycle with food and water ad libitum. Ambient housing temperature was maintained at $\sim 25.5^{\circ}\text{C}$ with 40–60% humidity. We used 31 mice for the Purkinje cell recordings for [Figures 1, 2, 3, 4, and 5](#) mice for the optogenetic experiments, 14 mice for the anatomical experiments.

METHOD DETAILS

Surgeries

For all behavioral and electrophysiological recordings, a custom-made magnetic pedestal was attached to the skull of the mouse above bregma using Super-Bond C&B (Sun medical, Moriyama, Japan) and a craniotomy was made over Crus1/Crus2. Surgical procedures were performed under isoflurane anesthesia (ISOFLUTEK 1000, Karizoo, Barcelona, Spain; 2–4% V/V in O_2). Mice were given 5 mg/kg carprofen (“Rimadyl”, Pfizer, New York, NY), 1 μg lidocaine (AstraZeneca, Zoetermeer, The Netherlands), 50 $\mu\text{g}/\text{kg}$ buprenorphine (“Temgesic”, Reckitt Benckiser Pharmaceuticals, Slough, United Kingdom) and 1 μg bupivacaine (Actavis, Parsippany-Troy Hills, NJ, USA). After three days of recovery, mice were habituated to the recording setup during at least 2 daily sessions of approximately 45 min. In the recording setup, mice were head-fixed with the pedestal and restrained ([Figure 1](#) top right).

Whisker movement recording and tracking

Videography was performed using a high-speed camera (acA640-750um, Basler Electric Highland, Illinois, USA) placed at ~ 50 cm above the mouse. The whisker movement was captured at a frequency of 1 KHz (1 frame/ms). The whisker movements were tracked as described previously⁴⁷ using the BIOTACT Whisker Tracking Tool¹⁰⁶ in combination with custom written code (https://github.com/elifesciences-publications/BWTT_PP). The whisker movements were described as the average angle of all trackable whiskers per frame.

Electrophysiology

Electrophysiological recordings were performed in awake mice using quartz-coated platinum/tungsten electrodes (2–5 M Ω , outer diameter = 80 μm , Thomas Recording, Giessen, Germany) placed in an 8x4 matrix (Thomas Recording), with an inter-electrode distance of 305 μm . Prior to recording, mice were lightly anesthetized with isoflurane to remove the dura, fix them in the apparatus and adjust all manipulators. Recordings in right Crus1/Crus2 at a minimal depth of 500 μm began at least 60 min after termination of anesthesia. The electrophysiological signal was digitized at 25 kHz, using a 1–6,000 Hz band-pass filter, 22x pre-amplified and stored using a RZ2 multi-channel workstation (Tucker-Davis Technologies, Alachua, FL). Spikes were detected offline using SpikeTrain (Neurasmus, Rotterdam, The Netherlands). A recording was considered to originate from a single Purkinje cell when it contained both CSs (identified by the presence of stereotypic spikelets) and SSs, when the minimal inter-spike interval of SSs was 3 ms and when each CS was followed by a pause in SS firing of at least 8 ms. A recording was considered to originate from a CNN based on stereotaxic coordinates and post-hoc electrolytic lesions performed in a subset of experiments. We accepted only those recordings during which the amplitude and the width of the spikes were constant over time. The recordings in which the amplitude or the width of more than three consecutive spikes exceeded three standard deviations above or below their average were considered unstable and excluded. In this way, any change in spike rate due to the instability of the recordings was avoided. When these criteria were satisfied, we considered them stable single-unit recordings, and those with a minimum recording duration of 40 s that comprehended whisker movements were selected for further analysis. In total 127 PCs and 73 DCN cells fulfilled these criteria. The PCs had an average duration of 349 ± 206 s of SD (range 49 s to 1039 s). The DCN cells had an average duration of 351 ± 228 s of SD (range 49 s to 1157 s). Often multiple cells were recorded simultaneously from the same mouse. The number of simultaneously recorded PCs varied between mice, but also within individual mice between the subsequent epochs of recordings. On average, in each mouse, at each moment of recording, 2.2 PCs were recorded simultaneously (range 0 to 9 cells). Out of the 200 neurons (PC and DCN) recorded and analyzed, we obtained 35 pairs of PC and DCN with an average duration of 283 ± 195 s of SD (range 51 s to 805 s).

Viral injections

AAV9-Syn-ChrimsonR-tdTomato and AAV5-hSyn-Cre-eGFP were obtained from UNC Vector Core. All viral vectors were aliquoted and stored at -80°C until used. To express ChrimsonR in climbing fibers, 50 μl of AAV9-Syn-ChrimsonR-tdTomato viral vectors was injected in the left inferior olive using the following coordinates: -2.9 mm posterior to lambda, 0.5 mm lateral, and 5.3 mm deep. After 6 weeks of incubation, a craniotomy (as described above) was performed on the contralateral cerebellar hemisphere (right side). For the anatomical tracing shown in [Figure 7](#), 80 μl of AAV-CAGsm-myc⁵⁵ was injected unilaterally at a depth of 600 μm in five mice. Two mice received the injection in the medial part of lobule Crus2 (-0.3 mm posterior to the horizontal fissure, 0.3 mm lateral to the vermal longitudinal sulcus) and the other three in the regions surrounding it: Medial Crus1 (+0.3 mm posterior to the horizontal fissure, 0.3 mm lateral to the longitudinal sulci), lateral Crus1/Crus2 (0.0 mm to the horizontal fissure, 2 mm lateral to the vermal longitudinal

sulcus), and lateral Crus 1 (–0.3 mm posterior to the horizontal fissure, 2 mm lateral to the vermal longitudinal sulcus). After a survival period of 10 weeks, the animals were sacrificed and their brains were processed for histology. For the anatomical tracing shown in [Figure S7](#), 50 μ l of AAV9-hSyn-FLEX-tdTomato was injected unilaterally into the cerebellar nuclei of 3 Gad2-ires-Cre and 3 VGluT2-ires-Cre mice. We used the following coordinates: –2.5 mm posterior to lambda, 2 mm lateral, and 2.3 mm deep. After a survival period of 6 weeks, the animals were sacrificed, and their brains were processed for histology.

Histology and microscopy

Animals were deeply anesthetized with isoflurane and intraperitoneal injection of pentobarbital sodium solution (50 mg/kg). They were perfused transcardially with saline, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.4). Brains were removed immediately and post-fixed for an hour in 4% PFA in 0.1 M PB at 4 °C. Fixed brains were placed in 10% sucrose overnight at 4 °C and then embedded in 12% gelatin-10% sucrose. After fixation in 10% formalin for an hour, the blocks were placed in 30% sucrose overnight at 4 °C. 40- μ m serial coronal sections were cut with a freezing microtome (SM2000R, Leica) and collected in 0.1 M PB. For immunofluorescence, sections were incubated subsequently with primary and secondary antibodies. All antibodies were titrated for working solution in a 2% normal horse serum-0.4% triton-0.1 M PBS solution. Tissue was incubated in primary antibodies at 4 °C overnight and in secondary antibodies at room temperature for 2 h. After each incubation with antibodies, sections were gently rinsed with 0.1 M PBS (10 min, 3 times) and subsequently mounted for microscopy. For all immunofluorescence sections, DAPI was used for general background staining. For fluorescence imaging, we took overviews of the brains with a 10x objective on a fluorescence scanner (Axio Imager.M2, ZEISS).

Optogenetic stimulation

LED photostimulation (wavelength = 595 nm, M595F2, Thorlabs, Newton, NJ, USA) was given by a high-power light driver (DC2100, Thorlabs, Newton, NJ, USA) through an optic fiber (400 μ m in diameter, Thorlabs, Newton, NJ, USA). The optic fiber was placed on the surface of Crus1/Crus2 in eight different locations. In a subset of experiments, a fiber optic was implanted (200 μ m in diameter, Thorlabs, Newton, NJ, USA) for direct stimulation of the inferior olivary region (coordinates: –2.9 mm posterior, 0.5 mm lateral, and 5.3 mm to lambda). Trials with stimulation at the surface of the cerebellar cortex and inferior olivary region were randomly intermingled with trials in which the same type of light (wavelength = 595 nm) was delivered near the mouse as a control for sensory responses to the light. The intensity of the light was calibrated so that only whisker movements were triggered when the mouse was at rest. Together with the relative short latency of ~20 ms, which is in line with that of for example triggering eye movements or limb movements with optogenetic stimulation of Purkinje cells,^{83,107} this allowed us to exclude the possibility that whisker movements were reflecting largely secondary effects following other non-whisking movements.

QUANTIFICATION AND STATISTICAL ANALYSIS

Anatomical maps

To visualize the distribution of features across Crus1/Crus2, we developed an anatomical heat map of the distribution of the Z-scored values obtained ([Figure 2C](#)). Since the electrophysiological recordings were performed using a grid of 8 x 4 electrodes (2 x 1 mm, placed always on the same type of craniotomy to cover Crus1/Crus2), we could retrieve the approximate location of each cell and plot the corresponding maximum Z-score on an 8 x 4 matrix. Linear interpolation was used to smooth the edges of adjacent patches to create the smooth map of feature distribution. Finally, the MATLAB function "imagesc" was used to obtain the heat map overlaid on a schematic of the craniotomy.

Statistics and visualization

The illustration of the mouse at the top right of [Figure 1A](#) was created with [BioRender.com](#). The heatmap of [Figure 1A](#) was obtained using the function "wcoherence" of the MATLAB wavelet toolbox (Wavelet Toolbox). This function also provides a matrix of the values of magnitude-squared coherence at each timepoint for a wide range of frequencies (from 0 to ~300Hz). The plots in [Figure 1C](#) show the mean coherence in between 3-30 Hz (which corresponds to the range of whisking frequencies in mice). Throughout the manuscript, we calculated how much the observed results differed by what could be expected by chance employing a bootstrap method that used random permutations of the actual data. For [Figures 1, 2, 3, and 4](#), the real CS timings were shuffled randomly to obtain data with identical structure and probability of bias such as accidental synchronization with the level of coherence. The shuffled data were used to calculate shuffled results for comparison. By repeating this process 100 times, we obtained a distribution of results that could be expected by chance. Then the mean and standard deviation of the random results were calculated at each time point and subsequently used to calculate the Z-score of the observed data. Z-scores >2 and <-2 as well as data points outside the 5th-95th percentile range were considered as exceeding chance probability. For [Figure 2](#), the method described above was used to calculate the Z-score of the local cross-correlation between left and right whisker movements. The local cross-correlation was calculated using a custom-code based on the MATLAB function "xcorr". The cross-correlogram was calculated for subsequent time windows of 25 ms and its maximum value was extracted and used for further analyses. Individual whisker protractions (single protraction, SP) in [Figures 3 and 4](#) were detected with a custom MATLAB code that detected the points of maximum and minimum of the filtered whisker signal (30 Hz low-pass filter). The segment movements with only positive velocity and an amplitude larger than 3 degrees were considered for this analysis. Protractions featuring a succession of two of those segments (i.e., double pumps) were not

included. To average protractions of different durations, we normalized the duration of each protraction to 1. As a result, all protractions are made to have the same start and end point and the only changing parameter is amplitude. Therefore, we analyzed phase and amplitude of the protraction, averaged across all protractions. The velocity of the protraction was calculated as the first derivative of the normalized protraction, allowing us to calculate velocity at each phase of the cycle. In [Figure 4](#), the individual protractions were divided into three groups based on the pre-CS ISSR. For each PC, we first calculated the differential of the SS times to obtain the inter-spike intervals. Then we calculated the reciprocal of each inter-spike interval ($1 / \text{inter-spike interval}$) to obtain the ISSR that we convolved with a mix of Gaussian filters (kernel size: 5 ms and 20 ms) obtaining a time series that fluctuates and represents the amount of SS at each time point. Finally, we interpolated the time series of the ISSR to attain a value at each ms (we interpolated it to obtain 1000 data points for each second of recording, which corresponds to the sampling frequency of the whisker data). To check that after this type of data transformation, the ISSR accurately represents the PC activity, we calculated the average values of the ISSR vector of each PC and compared it with the corresponding overall SSR (amount of SSs / duration of the recording). We used a time window of 5 ms prior to each CS, because the minimum inter-spike interval for SSs is ~ 4 ms, resulting in a maximum ISSR of ~ 250 Hz.¹⁰⁸ If 2 SSs are present in a time window of 5 ms, it means that at that time the maximum ISSR has been reached. Thus, the time window of 5 ms prior to the CS is a proper window to see if the SS frequency was maximum when the CS has occurred. Since we calculated the ISSR with 1 ms time resolution (as described above) we used the average value of the 5 ms prior to the CS as a measure of the ongoing ISSR at the time that each CS occurred. If the pre-CS ISSR was less than 0.5 SD below the overall ISSR, the CS-related SP was placed in the group 'low'. If the average pre-CS ISSR was within 1 SD of the overall ISSR, the CS-related SP was placed in the group 'middle'. If the pre-CS ISSR was higher than 0.5 SD above the overall ISSR, the CS-related SP was placed in the group 'high'. Only cells that displayed firing that fit into all three groups were considered. Note that the same grouping method has been used for the data after each random permutation of the CS timing. We compared whisker protraction with a high pre-CS ISSR with whisker protraction with the high ISSR, but without real CSs (but associated with a CS after shuffling randomly the CS timing). Thus, in this case, the Z-score expresses how different a protraction with a high pre-CS ISSR is from a protraction with a high ISSR, but without a CS. For [Figures 5G](#) and [5H](#), each mouse received 50 light stimulations for each of the 8 locations. The cross-correlation between left and right whisker movement was calculated at an individual trial level using a sliding window of 25 ms. The movement was considered symmetrical when the cross-correlogram within each 25 ms window had a positive peak and asymmetrical when the peak was negative (in case peaks of opposite sign were present we selected the most extreme value). This method is the same as described above for [Figure 2C](#). In [Figure 5](#), however, it was not possible to shuffle randomly the CS timing, because they were induced by the optogenetic stimulation. The Z-score has been calculated by comparing the cross-correlation of the trials of each location with the cross-correlation of trials taken randomly from all locations. Groups of 50 trials were selected randomly from all the trials of all 8 stimulated locations and the resulting cross-correlation was calculated. This process was repeated 100 times producing a distribution of level of cross-correlation of intermingled trials. Then, the Z-score of cross-correlation of each location was calculated using the mean and the SD of the 100 permutations with cross-correlation of intermingled trials. Thus, in [Figure 5](#), the Z-score represents how well the left and right whisker movements induced in each of the 8 locations are cross-correlated.

Coherence-CNN spikes matrix of correlation

The magnitude-squared coherence (MSC) at each timepoint was obtained using the function "wcoherence" of the MATLAB wavelet toolbox (Wavelet Toolbox). The maximum value of MSC between 3–30 Hz as well as the instantaneous CNNs spike firing rate was used for the matrix of correlations as described before.^{32–34} In short: spike density functions were computed for all epochs of whisker movements by convolving spike occurrences across 1 ms bins with an 8 ms Gaussian kernel. For cell groups, data were standardized for each cell for each correlation, and then pooled. The spike-whisker Pearson correlation coefficient R was calculated in bins of 10 ms, resulting in a 60x60 R-value matrix showing correlations for -200 to 400 ms around the onset of the whisker movement. The R-value on the diagonal of the matrix was used to assess significance. If any of the R-values after movement onset (from 0 to 300 ms) exceeded two SDs of the R-values before movement onset (from -200 to -100 ms) the correlation was considered significant.