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Shared cortex-cerebellum dynamics in the execution and learning of a motor task

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SUMMARY

Throughout mammalian neocortex, layer 5 pyramidal (L5) cells project via the pons to a vast number of cerebellar granule cells (GrCs), forming a fundamental pathway. Yet it is unknown how neuronal dynamics are transformed through the L5→GrC pathway. Here, by directly comparing premotor L5 and GrC activity during a forelimb movement task using dual-site two-photon Ca²⁺ imaging, we found that in expert mice, L5 and GrC dynamics were highly similar. L5 cells and GrCs shared a common set of task-encoding activity patterns, possessed similar diversity of responses, and exhibited high correlations comparable to local correlations among L5 cells. Chronic imaging revealed that these dynamics co-emerged in cortex and cerebellum over learning: as behavioral performance improved, initially dissimilar L5 cells and GrCs converged onto a shared, low-dimensional, task-encoding set of neural activity patterns. Thus, a key function of cortico-cerebellar communication is the propagation of shared dynamics that emerge during learning.

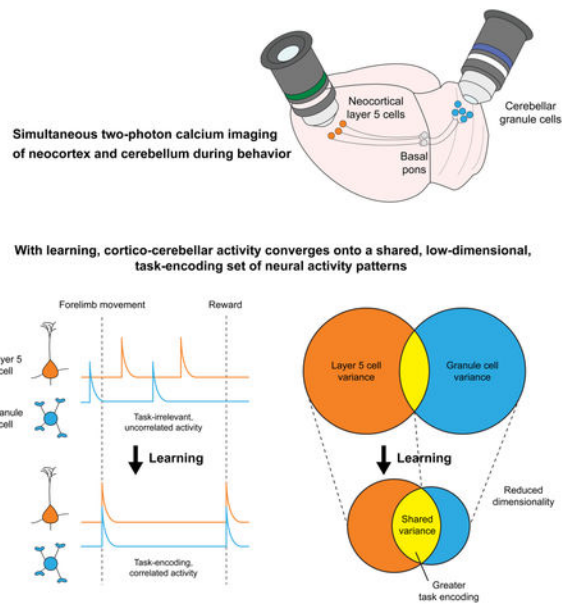
Graphical Abstract

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Author Contributions M.J.W. and L.L. designed the experiments, M.J.W. performed experiments and analyzed the data with help from T.H.K. and N.D.N. T.H.K. designed the multi-access optical strategy and performed Ca²⁺ data processing. J.K. and S.G. performed simulations and data analysis. M.J.S. contributed imaging hardware, software, and expertise. L.L. supervised the project. All authors contributed to writing the manuscript.

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In Brief

Simultaneous recordings of ensembles of individual neurons in the neocortex and cerebellum provide a view of how these two brain regions learn together

INTRODUCTION

Mammalian brain evolution has maintained a remarkably conserved ~4:1 ratio of total neurons in the cerebellum to that in neocortex, with these two structures containing ~99% of neurons in the human brain (Barton and Venditti, 2014; Herculano-Houzel, 2010). Cerebellum and neocortex are also densely interconnected: most neocortical regions send layer 5 (L5) projections to the pontine nuclei, which provide the largest input to the cerebellum through granule cells (GrCs) (Brodal and Bjaalie, 1997; Kelly and Strick, 2003; Suzuki et al., 2012). However, little is known about either the propagation of cortical dynamics into the GrC layer, or how properties of cortico-cerebellar communication develop with learning.

GrC anatomy is highly distinctive: each GrC receives only four inputs, called mossy fibers, which are fixed during development and can originate from neocortex via the pontine nuclei, as well as from the brainstem and spinal cord (Huang et al., 2013; Sillitoe et al., 2012). Moreover, different GrCs are unlikely to share the same set of four inputs. Therefore, any individual signal originating in L5 might recombine with three other disparate mossy fibers in a given GrC, and the vast number of GrCs (more than half of all neurons in the brain) could permit many distinct input recombinations. This basic, conserved anatomical feature is thought to allow the GrC layer to produce outputs highly distinct from those of cortex (Albus, 1971; Babadi and Sompolinsky, 2014; Billings et al., 2014; Cayco-Gajic et al., 2017; Chabrol et al., 2015; Litwin-Kumar et al., 2017; Marr, 1969).

Despite these anatomical clues, studies have yet to detail the functional properties of L5-GrC transmission and its evolution with learning. This stems from a few technical hurdles. First, recording granule cells is challenging due to their small size and high packing density, with recordings from ensembles of granule cells in behaving animals only recently achieved via two-photon Ca^{2+} imaging (Giovannucci et al., 2017; Knogler et al., 2017; Wagner et al., 2017). Second, simultaneous single-cell-resolution recordings have not yet been obtained from L5 cells and GrCs. As a result, prior studies of cortico-cerebellar interaction have not observed L5-GrC signal transmission and its evolution during learning. Here, we devised a strategy for simultaneous chronic two-photon imaging of premotor cortical L5 neurons and cerebellar GrCs, and uncovered surprisingly shared cortico-cerebellar dynamics that emerged as animals gained expertise on a forelimb movement task.

RESULTS

Simultaneous Imaging of Neocortex and Cerebellum in Behaving Mice

To characterize disynaptic projections from the neocortex to the cerebellum in mice, we performed projection-based monosynaptic retrograde rabies tracing (TRIO; Schwarz et al., 2015) to identify cortical neurons presynaptic to pontine neurons that project to the cerebellar cortex. We found neurons from nearly every cortical region were presynaptic to pontine neurons that project to the dorsal surface of the cerebellum (Figure S1), similar to previous reports in rats and monkeys using polysynaptic rabies tracing (Kelly and Strick, 2003; Suzuki et al., 2012). We focused on the premotor cortex, given the importance in theoretical models of cortical transmission of motor plans to the cerebellar cortex (Moberget and Ivry, 2016).

We devised a strategy to simultaneously monitor activity of premotor L5 cells (*Rbp4-Cre+* pyramidal neurons) and the cerebellar input layer (GrCs) with single-cell resolution. We adapted a custom two-photon microscope that enabled imaging of two distant brain areas via a pair of mechanically articulated optical arms, each equipped with its own microscope objective lens (Lecoq et al., 2014) (Figure 1A, left; Figure S2). To image premotor cortex at the rostral forelimb area, we used a micropism for better optical access to layer 5b, which is enriched for subcortically-projecting pyramidal neurons. We also placed a cranial window over cerebellar lobules VI, simplex, and crus I (Wagner et al., 2017), regions that are forelimb-related and receive heavy inputs from the pontine nuclei (Huang et al., 2013; Suzuki et al., 2012) (Figure 1A, right). We used transgenic mice that expressed the genetically-encoded Ca^{2+} indicator GCaMP6f (Chen et al., 2013; Madisen et al., 2015) in both L5 cells and GrCs. Together, these methods allowed simultaneous 30-Hz two-photon imaging of somatic Ca^{2+} activity of 73 ± 7 premotor L5 cells and 86 ± 7 cerebellar GrCs (mean \pm SEM across $n=28$ imaging sessions in 10 mice) (Figure 1B and Movie S1). Due to Ca^{2+} indicator kinetics, the GCaMP6f transients in our imaging data likely correspond primarily to multiple spikes (Chen et al., 2013; Giovannucci et al., 2017) (STAR Methods). Thus, our recordings are more attuned to sustained activity, as observed in cortex during the planning or delay periods of motivated behaviors (Li et al., 2015), than to individual spikes. We therefore designed a movement planning task with the potential to engage sustained neural signaling.

Our task required mice to make a sequence of two perpendicular motions in a virtual track (STAR Methods): a 6-mm forward motion followed by a 6-mm lateral motion to the left or to the right, with reward delivered after a delay period of 1 s. Left and right trials occurred in alternating blocks of 40 trials, without a cue (Figures 1C–E). The common forward motion preceding both left and right turns implied that different neural states prior to turning likely reflected different movement sequence plans. For analyzing behavior, we classified “pure” turn trials as those in which the mouse did not push the handle in the incorrect lateral direction by more than 500 μm at any point during either the forward or lateral motion segments—a strict criterion to identify correctly planned motions. Trials in which the mouse exceeded this threshold were scored as error trials, regardless of the mouse’s paw motion subsequent to the erroneous motion (in some cases, the mouse “recovered” to successfully execute the correct motion, Figure 1D, while in other cases, it exceeded a physical threshold beyond which the trial automatically terminated, STAR Methods). After training (~3 weeks, ~30 min/day), expert mice executed pure turns on $60\pm 3\%$ of attempts, with movements spanning ~400 ms in total (Figure 1F). Optogenetic manipulations demonstrated that both the cortical and cerebellar regions that we imaged were critically necessary for task execution (Figure S3A–G). By examining single-cell activity traces, we found that both L5 cells and GrCs often appeared preferentially active during trials of one turn direction (Figure 1G).

Similar Task Representations in L5 Cells and GrCs in Expert Mice

We first characterized neural representations of the motor task in expert mice. To identify task-locked activity of each cell, we aligned its time-varying fluorescence on all trials to turn onset, and then computed the average across trials (separately for pure left and pure right turns). We often observed L5 cells with direction-preferring responses both during and substantially earlier than the onset of movement (Figures 2A and 2B, top). This is consistent with other planning tasks, in which premotor cortex activity precedes upcoming movements (Li et al., 2015; Shenoy et al., 2013). In addition to movement-locked signals, we computed time-varying trial-averaged fluorescence aligned to reward delivery and found that many L5 cells responded selectively prior to or during reward consumption, often preferentially following one turn direction (Figures 2C and 2D, top).

We next examined the trial-averaged activity of GrCs, which exhibited response profiles with selectivity similar to that of L5 cells (Figures 2A–D, bottom). While we have previously reported reward-related signals in GrCs (Wagner et al., 2017), to our knowledge this is the first report of movement-planning-related signals in GrCs. To quantify the prevalence of different responses in L5 and GrC ensembles, we defined a set of behavioral regressors that each indicated a key task event: pre- and post-turn and pre- and post-reward, separately for left and right pure turn trials (Figure 2E). Cells were considered responsive to a task event if the corresponding regression coefficient was significant, and direction-preferring if the coefficient was significantly larger for one turn direction (STAR Methods). Overall, similar proportions of active L5 cells and GrCs were direction-selective during each task phase (Figures 2F), which was surprising given that input recombination in the GrC layer is thought to generate activity profiles distinct from those of the neocortex (Marr, 1969). However, while active L5 cells and GrCs contained broadly similar responses, the

populations differed in the fraction of visible GCaMP-expressing cells *without* any detectable activity, suggesting differential recruitment of L5 cells vs. GrCs by our task. Of the cells with visible baseline fluorescence, 55% of GrCs and 18% of L5 cells were undetected by the cell-extraction algorithm, which only identifies neurons with activity. Manual analysis confirmed that such undetected neurons had near-zero Ca^{2+} event rates (STAR Methods). Since neurons that we did not extract were inactive (possibly due to spiking levels below detection threshold), L5 cells and GrCs hereafter denote the set of all extracted neurons.

To further assess and compare motor planning dynamics in L5 and GrC ensembles, we defined a metric to discriminate turn direction using each population's activity prior to turn onset. On each trial, we computed the time-varying difference between the average activity of all pre-left-turn- and of all pre-right-turn-preferring L5 cells (Figure 2G, left) and GrCs (Figure 2G, right, normalized to range from -1 to 1 ; cells identified from regressions in Figure 2F). Direction discrimination rose at similar rates in both L5 cells and GrCs prior to pure turns, but was absent prior to motion on error trials. Hence, pre-turn L5 cells and GrCs exhibited similar motor planning dynamics.

We next used linear regression analysis to directly compare the similarity of active L5 and GrC ensembles. We found that GrC ensembles were as accurate as L5 ensembles at reproducing the trial-averaged time-varying activity of individual L5 cells (Figure 2H), indicating that most activity profiles in L5 were recoverable in the GrC layer. We also quantified the overall diversity of ensemble activity using principal components analysis (PCA). We performed PCA across all cells, using each cell's concatenated activity on all movements (pure turns and errors). Thus, for each imaging session, we performed one PCA on a $(T \times N)$ -by- C matrix, where T is the number of trials, N is trial duration (-2 to 2 s relative to turn onset), and C is the number of cells. The resulting principal components are linearly independent activity patterns that account for the most variability across neurons. We found that the number of principal components needed to explain a given fraction of population activity in GrCs was slightly lower than in L5 cells (Figure 2I). Together, these results indicate that L5 cells and GrCs encoded the task similarly, had common trial-averaged response profiles, and exhibited comparable response dimensionality.

Highly Correlated Single-trial L5-GrC Activity in Expert Mice

In addition to sharing similar task representations in their trial-averaged activity, L5-GrC pairs often exhibited strong single-trial correlations (Figure 3A). We sought to quantify correlations within and across L5 and GrC populations. Although correlation magnitudes depend on how they are measured (Cohen and Kohn, 2011), all measurement factors were common to L5 and GrC recordings. Thus, the *inter*-areal and *intra*-areal correlations can be directly compared. We used the concatenated single trial activity of each cell [the $(T \times N)$ -by- C matrix described above] and computed the matrix of pairwise correlation coefficients between every pair of columns of the matrix. We first characterized each neuron's correlation to other neurons via its "best-match" partner cell (Figure 3B). Remarkably, overall L5-GrC correlation magnitudes were nearly as high as those between different L5 cells in our small imaging fields (Figures 3B, 3C). L5-GrC correlations were also consistent

across the cerebellar lobules we imaged (Figure 3D). Correlations between GrCs were even higher than L5-L5 or L5-GrC correlations (Figures 3B, 3C; Figure S3H). Analysis of the distribution of correlations among *all* cell pairings yielded similar results (Figure S3I).

Control analyses suggest that L5-GrC correlations were contributed substantially by shared trial-to-trial variability, rather than resulting only from common task tuning (Figure S3J, K). To exclude the possibility that correlations result from systematic factors, we performed simultaneous imaging of GrCs and the orbitofrontal cortex (OFC), where the density of L5 cells that project to cerebellum via pons is similar to that of premotor cortex (Figure S1). Our OFC L5-GrC data shared the same systematic factors as our premotor L5-GrC data: transgenic mice, Ca²⁺ indicator kinetics, genetically defined class of L5 cells, and motor task. But in contrast to our premotor L5-GrC data, OFC L5-GrC correlations were substantially weaker (Figures S3L–O). Thus, no systematic factors or artifacts can account for high premotor L5-GrC correlations. Taken together, high correlations between premotor L5 cells and GrCs demonstrate shared dynamics.

GrCs Exhibit More Ca²⁺ Events and Greater Reliability than L5 Cells

High single-trial correlations between GrCs and premotor L5 cells demonstrated faithful recapitulation of cortical dynamics in the GrC layer. To identify more subtle differences between GrCs and L5 cells, we analyzed correlated L5-GrC pairs in greater detail. Even for highly correlated L5-GrC pairs, activity in the GrC and L5 cell still occasionally differed. We found that L5-GrC discrepancies frequently resulted from Ca²⁺ events in the GrC that were missing from the L5 cell (Figure 3E). Overall, for highly correlated L5-GrC pairs (defined arbitrarily as $r > 0.4$), a significantly greater proportion of GrC events were present only in the GrC (“GrC-only” events), compared to the proportion of L5 events that were present only in the L5 cell (“L5-only” events, Figure 3F).

To determine the behavioral significance of GrC-only versus shared L5-GrC events, we compared the temporal distribution of the two event types relative to forelimb movement. Most often, GrC-only events occurred at similar times during the trial as shared L5-GrC events (Figure 3G). This indicated greater GrC reliability relative to similar L5 cells, potentially resulting from pontine integration of similarly-tuned L5 neurons. Less frequently, we observed GrCs for which GrC-only events were temporally distinct from shared L5-GrC events (Figure 3H), potentially reflecting GrC multiplexing of disparate input signals. Overall, GrCs often exhibited more activity with more reliable signaling than the L5 cells to which they were correlated (Figure 3I). Thus, while L5 cells and GrCs share similar dynamics, GrC encoding is of greater fidelity, suggesting that pontine integration may reduce noise. Simulations indicated that the shared L5-GrC dynamics in our data are challenging to explain if GrC output combines substantial contributions from multiple mossy fiber inputs, but follow naturally if the output of some GrCs is dominated by a single input, such as a task-related signal originating in cortex (Figure S4).

Pontine Contribution to L5-GrC Dynamics

To verify that cortico-cerebellar transmission contributes to L5-GrC correlations and GrC task representations, we expressed inhibitory opsins in the basal pontine nuclei (Figure 4A).

In expert mice ($n=10$), we imaged GrCs while photoinhibiting pontine neurons on a random 20% of trials (Figures S5A–K). In a small subset of GrCs, turn direction-preferring responses were abolished by pontine photoinhibition (Figures 4B, 4C). Inhibition was equally common in cells of each response type (Figure S5L). In total, 10% of GrCs were inhibited and 10% were disinhibited during pontine inhibition (Figures 4D, 4E, S5M, and S5N), the latter likely due to reduced inhibition from Golgi cells (Billings et al., 2014).

In six mice, we performed simultaneous premotor L5 and GrC imaging in conjunction with optogenetic inhibition of the pons. Pontine inhibition lowered L5-GrC correlations (Figure 4F) and decreased the fraction of GrC activity explained by L5 using linear regression (Figure 4G). These data likely substantially underestimate the effect of pontine input to GrCs, due in part to incomplete viral coverage of the pontine nuclei (Figure S5O). As a result, L5 activity was largely unaffected (Figure S5P) and behavior was unchanged during the random 20% interleaved pontine inhibition trials (Figure S5Q). Thus, changes in GrC activity were most likely due to the direct effects of diminished pontine input to GrCs, rather than indirect consequences of altered cortical activity or behavior. (However, behavioral performance was degraded during an alternative paradigm employing continuous inhibition for two 40-trial blocks of movements; Figure S5R). While there may also be contributions from common input or cerebello-cortical feedback (Gao et al., 2016), these data indicate that pontine transmission contributes to GrC task encoding and to L5-GrC correlations.

Common L5 and GrC Task Representations Emerge Concurrently over Learning

What is the origin of shared cortico-cerebellar dynamics observed in expert mice? To address this question, we tracked the activity of individual L5 cells and GrCs over the 2–3 week course of task learning (Figures 5A and 5B). Our training procedure began with an initial period (3–7 days) without imaging during which mice learned the basic task structure by performing forward-only movements in a linear track for reward. Chronic imaging began on the first day in which mice were exposed to the movement sequence task. Early in learning, both L5 cells and GrCs often had activity time-locked to movement or reward without distinguishing left- from right-turn trials. Over time, these cells lost their responsiveness to one turn-direction selectively (Figures 5C and 5D). Equally common were cells that were time-locked to a particular phase of the task and with strong direction preference late in learning, but which were not time-locked at that phase early in learning (Figures 5E and 5F).

To quantify these trends, we used two methods to assess the neural encoding of behavior. First, we examined behavioral encoding by individual cells, using linear regression of the single-trial fluorescence of each individual L5 cell or GrC onto the set of behavioral regressors from Figure 2E. We thereby identified all cells which, late in learning, had significant direction-preference during a particular phase of the task. We found that, earlier in learning, such cells were generally either not responsive at that time, or responsive but not direction-selective (Figure 5G). Overall, substantially more neurons exhibited direction-preference late in learning (Figure 5H). Moreover, these regressions more accurately reproduced each cell's activity after learning (Figure 5I), indicating stronger relationships between neural activity and behavior.

Second, we assessed the behavioral information conveyed by the L5 and GrC neural ensembles. We defined “behavioral signals” that selectively indicated either movement or reward on either left or right turn trials (Figure S6A, left). We then used the concatenated single-trial population activity of either L5 or GrC ensembles to reproduce each of these behavioral signals in turn via separate linear regressions (Figure S6A, right), and tabulated the mean accuracy of these regressions (R^2). This analysis demonstrated that both L5 and GrC ensembles encoded more behavioral information after learning (Figure 5J). Moreover, the fidelity of GrC and L5 ensemble behavioral encoding covaried across imaging sessions (Figure 5K). Thus, task encoding emerges concurrently in L5 cells and GrCs during learning.

In addition to *increasing* the prevalence of task-encoding neurons, learning also *decreased* the overall diversity of activity among different neurons in the L5 and GrC ensembles. For each imaging session, we performed PCA across cells on single-trial activity from all movements (as in Figure 2I). We tabulated both the variance explained by the top 10 PCs, as well as the number of PCs required to explain 50% of variance in ensemble L5 or GrC activity in each session. Over learning, both parameters indicated reduced dimensionality in both L5 cells and GrCs (Figures 5L and 5M). Trial-averaged response profiles similarly became lower dimensional (Figure S6B). Thus, L5 cells and GrCs together exhibit increased task encoding and reduced response diversity during learning.

Cortico-cerebellar Correlations Rise over Learning

Are the strong L5-GrC correlations a product of connectivity established during development, or produced during learning? To address this question, we first identified L5-GrC pairs that were highly correlated on the final day of imaging (arbitrarily defined as pairs with $r > 0.4$). We found that despite robust last-day correlations, these pairs were less correlated earlier in learning (Figures 6A; S6C). To exclude the possibility that this resulted simply from random fluctuations in correlations caused by the passage of time, we similarly identified L5-GrC pairs that were highly correlated early in learning ($r > 0.4$), which were less common. We found that such pairs tended to remain substantially correlated late in learning (as compared to the initial correlations of pairs with high last-day correlations; Figure 6B). In addition, when considering *all* cells, correlation magnitudes similarly rose with learning (Figure S6D, E). Increased correlations were due both to more similar trial-averaged activity patterns and to greater shared trial-to-trial variability (Figures S6F, G). Increased correlations were also not caused by increased Ca^{2+} event rates, which fell slightly over learning (Figure S6H). Moreover, analysis of temporal lags in the computation of cross-correlations between all L5 cells and GrCs demonstrated that neurons also became more temporally aligned (Figure S6I). Consistent with increasing pairwise correlations, population L5 activity more accurately reproduced single-trial activity of individual GrCs via linear regression after learning (Figure 6C). Taken together, these data demonstrate that learning promotes L5-GrC single-trial correlations.

The rise in both *inter*-areal (L5-GrC) and *intra*-areal (GrC-GrC and L5-L5) correlations over learning suggested that the two ensembles had converged onto a shared low-dimensional space of activity patterns. To quantify this, we performed a reduced rank regression between

L5 cells and GrCs, which attempts to find the lowest-dimensional projection of L5 ensemble activity needed to reproduce the most possible GrC ensemble activity. This analysis confirmed that, compared to early learning, L5 ensembles in expert mice explained more GrC activity while using a lower-dimensional projection of their ensemble activity (Figure 6D).

We next investigated whether increased coupling between lower-dimensional L5 and GrC activity involved the emergence of mutually correlated groups of L5 cells and GrCs. In support of this, we found that GrCs that developed stronger correlations with the rest of the GrC ensemble over learning were substantially more likely to also exhibit increased correlation with L5 cells (Figure 6E). To explicitly identify mutually correlated groups of GrCs and L5 cells that emerged over learning, we performed k-means clustering analysis on the changes in population pairwise correlation coefficients (Figure 6F, top; clustering performed on the difference in correlations between the first and final days of imaging). Cell groups identified to have undergone coherent changes in correlation during learning contained substantial numbers of *both* L5 cells and GrCs (Figure 6F, bottom). We used mutual information to quantify the amount of information about cell type (L5 or GrC) provided by the cluster membership and found little tendency for clusters to segregate by cell type (Figure 6G). Taken together, these data suggest that over learning, groups of initially dissimilar L5 cells and GrCs converge together onto shared activity patterns.

Evolution of L5-GrC Dynamics Parallels Behavioral Improvement

To assess how emergent cortico-cerebellar dynamics related to improved behavioral performance over the multi-week learning process, we first quantified behavioral learning. We found that pure turns as a fraction of all trials rose over learning (Figure 7A, left). Kinematically, the average time to execute a movement decreased (Figure 7A, middle), due to faster transitions between the forward and lateral motions (Figure 7A, right; Figure S7A, S7B). Thus, mice planned more continuous movement sequences after learning. We summarized behavioral performance by the pure turn fraction and compared its session-by-session changes to the simultaneously acquired neural activity. Over weeks of learning, pure turn fraction covaried with behavioral encoding in both L5 and GrC ensembles (Figures 7B and 7C, assessed by the fidelity of linear regression to behavioral signals as in Figure 5J). In addition, L5-GrC coupling (measured by the accuracy of linear regression of single-cell GrC activity onto the L5 population activity, as in Figure 6C) also covaried with behavioral performance gains across imaging sessions (Figure 7D). Thus, behavioral learning is a key factor in the emergence of shared L5-GrC dynamics.

Shared Cortico-cerebellar Dynamics Reflect A Learned Circuit State

The parallel emergence of shared L5-GrC dynamics and improved behavioral performance may reflect different potential relationships between neural activity and behavior. In one possible scenario, neural representations could be *fixed with respect to motor output*. In this case, apparent increases in neural correlations over learning simply reflect more cohesive motor output. For example, more coherent activation of different muscles might cause the activity of neurons that represent distinct variables to appear more correlated. Alternatively, learning could recruit L5 cells and GrCs into more coherent dynamics through synaptic

changes. The first hypothesis predicts that cortico-cerebellar correlations should covary with trial-by-trial variations in motor performance within a single imaging session, rather than learning *per se*. By contrast, the circuit plasticity hypothesis predicts that L5-GrC correlations are determined by the state of learning in the circuit, which evolves slowly over days.

To distinguish between these hypotheses, we leveraged trial-to-trial variability in motor output during individual imaging sessions. We identified the trials most or least kinematically similar to the average pure turn trajectory in each imaging session (Figure 7E). We tested two predictions. First, in late learning, we found that L5-GrC correlations during least consistent trials were no different than during the most consistent trials (Figure 7F and S7C). Thus, trial-to-trial variations in motor output do not significantly alter cortico-cerebellar correlations. Second, we compared the *least consistent* trials *late* in learning to the *most consistent* trials in *mid*-learning. Despite greater motor stereotypy, the most consistent mid-learning trials exhibited substantially weaker L5-GrC correlations than did the least consistent late-learning trials (Figure 7F and S7C). Together, these data support the interpretation that L5-GrC correlations are produced by plastic circuit changes over the multi-week learning process: neural correlations are largely unchanged by trial-by-trial fluctuations in kinematic stereotypy within a single imaging session. These conclusions also held when we restricted our analysis to the set of neurons consistently tracked throughout learning (Figure S7D). Thus, our data suggest that the emergence of shared L5-GrC dynamics reflects plastic circuit changes that increase the prevalence of correlated task-encoding activity patterns across both populations.

DISCUSSION

By performing the first simultaneous recordings of neocortical layer 5 projection neurons and cerebellar granule cells in behaving mice over learning, we found that as animals learned, L5 and GrC ensembles converged onto increasingly shared, low-dimensional, and task-encoding activity patterns (Figure 7G). These data indicate that, although GrC anatomy permits diverse signal recombinations, a key outcome of learning in the cortico-cerebellar pathway is in fact increasingly similar dynamics in cortex and cerebellum. As a result, task-related L5 dynamics are faithfully recapitulated, rather than extensively transformed, in the GrC layer in expert mice.

Discrepancies with Dimensionality Expansion Theory and Potential Resolutions

At a basic level, GrC activity that is dominated by low dimensional, task-encoding L5 dynamics in expert mice differs from frameworks emphasizing GrC dimensionality expansion (Albus, 1971; Fujita, 1982; Marr, 1969). However, there are several caveats. First, dynamics and correlations will vary by the timescale of analysis (Cohen and Kohn, 2011; Kadmon and Sompolinsky, 2015), which in our data is set by the relatively slow GCaMP kinetics. While results may differ on faster timescales, our findings of increased correlations over learning are likely to remain similar. Second, we note that ~50% of GrCs and ~20% of L5 cells exhibited near-zero Ca^{2+} activity. While inactive cells cannot significantly increase the dimensionality of activity in our task, as they contribute little additional variance (STAR

Methods), they do suggest a larger reservoir of representational capacity in the GrC network, which is an important aspect of classical theory.

Finally, while the classical theory of granule cell function (Albus, 1971; Marr, 1969) focused on dimensionality expansion, a modern reanalysis of this theory indicates that maximal dimensionality expansion is not always preferable, as such expansion alone can also amplify input noise (Babadi and Sompolinsky, 2014; Kadmon and Sompolinsky, 2016; Litwin-Kumar et al., 2017). Thus, noise reduction is a theoretically predicted requirement for the cortico-cerebellar pathway. Our work supports this prediction, as GrCs in expert mice were often more reliable than L5 neurons (Figure 3). From this perspective, GrCs may sacrifice dimensionality expansion to avoid amplifying cortical noise in our task. A direction for future study would be to determine how L5-GrC correlations and dimensionality depend on task complexity (Gao and Ganguli, 2015).

Mechanistic Implications

Our data show that individual L5 cells and GrCs share similar activity in expert mice. Our simulations suggested that these findings were most consistent with a scenario where, for a subset of GrCs, activity is dominated by the input from just one mossy fiber (Figure S4), likely as a result of learning. This is surprising in light of GrC anatomy (see Introduction). On the other hand, the existence of inactive GrCs in our data may imply that different GrCs operate in qualitatively different regimes: classical sparsely active coincidence detectors (Chadderton et al., 2004) versus densely active relays of cortical dynamics, with transmission modes potentially modulated through plasticity (Gao et al., 2012). This segmentation of granule cell activity may have important effects on computation in downstream Purkinje cells (Galliano et al., 2013), which is an interesting subject for future study.

An additional key feature of our learning data is that directional selectivity emerged over learning in previously non-selective L5 cells and GrCs, seemingly at random (Figure 5). This observation suggests that L5-pons transmission is itself plastic (Figure S4). Specifically, it is likely that GrCs inherit direction selectivity from direction-selective mossy fiber inputs, since each GrC receives a fixed set of only four mossy fiber inputs. Thus, pontine neurons likely also transmit direction-selective signals. If, over learning, direction selectivity emerges randomly among different L5 cells, pontine neurons would need to adaptively reweigh different L5 inputs as they evolve, in order to avoid mixing away L5 selectivity before transmission to GrCs. Thus, a plastic cortico-pontine pathway may aid in the selection and denoising of cortical representations, before expansion in the GrC layer (STAR Methods).

New Perspective on Cortico-cerebellar Communication: Shared Dynamics Emerge with Learning

Our premotor L5 cortical data are broadly consistent with studies demonstrating that cortical neurons develop more stereotyped task-locked responses during learning (Peters et al., 2014). Other frontal cortical regions—similarly to the frontal premotor region we studied—have been found to exhibit relatively low dimensional activity that mimics task complexity,

in contrast to higher dimensional sensory areas (Brincat et al., 2018; Inagaki et al., 2018). Theoretical analysis suggests that, whereas machine learning algorithms often involve high-dimensional representations, low dimensionality—especially in simpler tasks—may be a fundamental outcome of learning in neocortical circuits, potentially increasing robustness to noise and aiding pattern completion (Denève et al., 2017).

Our simultaneous L5-GrC recordings expand these concepts by demonstrating that the cerebellum is strongly coupled into this learning process, as low-dimensional cortical dynamics that emerge with learning extend with striking fidelity into the cerebellar GrC input layer. This finding has important implications for the larger recurrent cortico-cerebellar network, in which cerebellar output returns to cortex via thalamus (Kelly and Strick, 2003). Recent work suggests that projections from the cerebellar nuclei to the neocortex are required for sustained cortical activity (Chabrol et al., 2018; Gao et al., 2018). Truly sparse GrC representation as classically predicted might prevent propagation of cortical dynamics through the cerebellar circuit. By contrast, if many GrCs faithfully transmit prominent cortical signals, they may more effectively shape downstream Purkinje cell and cerebellar nuclei firing patterns that ultimately feedback to cortex. Our findings that L5 and GrC ensemble dynamics co-evolve during learning support the notion that reciprocal interactions between cortex and cerebellum may underlie the learning process. Overall, our data suggest that it will be critical to study cortex and cerebellum as a joint dynamical system to fully understand the contributions of each to behavioral learning and performance.

STAR*Methods

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Liqun Luo (lluo@stanford.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice—All procedures followed animal care and biosafety guidelines approved by Stanford University’s Administrative Panel on Laboratory Animal Care and Administrative Panel on Biosafety in accordance with NIH guidelines. To express the Ca²⁺ indicator GCaMP6f (Chen et al., 2013) in cerebellar GrCs and neocortical layer 5 pyramidal cells, we used the Cre- and tTA-dependent GCaMP6f transgenic mouse line *Ai93* (*TRE-lox-stop-lox-GCaMP6f*) (Madisen et al., 2015). We crossed the *Ai93* mouse to a Cre-dependent tTA mouse *ztTA* (*CAG-lox-stop-lox-tTA*). In parallel, we crossed *Math1-Cre*, to obtain expression in granule cells as described previously (Wagner et al., 2017), to *Rbp4-Cre*, which in the cortex is expressed mainly in layer 5 pyramidal neurons (Gerfen et al., 2013). Mice were aged 6–16 weeks at the start of experimental procedures and were in good health. Except in the cases indicated, in which animals contributed to multiple datasets, mice were not used in previous surgical or experimental procedures. Prior to their training on the tasks used to generate the datasets in this study, mice were naïve to the behavioral task; mice used to study learning were naïve to the movement planning task, while mice that were only studied in the expert state had previously undergone training as described in the “Behavior” section below. We used a total of 24 *Ai93/ztTA/Math1-Cre/Rbp4-Cre* quadruple transgenic

mice (9 females and 15 males on mixed genetic backgrounds) for all experiments. In expert mice, behavioral performance was similar for male and female mice (pure turn fraction, $p = 0.76$, Wilcoxon rank sum test, $n = 29$ and 17 sessions from male and female mice). We also confirmed that pairwise correlations among granule cells, a central statistic of our findings, were similar in expert male and female mice ($p = 0.24$, $r = 0.43 \pm 0.01$ and 0.46 ± 0.02 in 29 and 17 sessions from male and female mice). 10 contributed to premotor cortex / cerebellum dual site imaging data of Figures 1–3. Of these, 7 were imaged repeatedly over the course of task learning (Figures 5–7), and of those 7, in 4 we were able to track the same neurons repeatedly until mice had mastered the task. 6 premotor / cerebellum dual-site mice, along with 4 cerebellum-only imaging mice, contributed to the pons-inhibition data (Figure 4). Of these 10 total, 7 underwent inhibition via eNpHR3.0 and 3 via iC++. 6 mice contributed to orbitofrontal cortex (OFC) / cerebellum dual site imaging data (Figure S3). 2 mice contributed lateral Crus II GrC imaging data in Figure S3. In addition, 12 wild-type C57/bl6 mice were used for tracing studies in Figure S1, and 3 double-transgenic *Gad2-Cre* (Taniguchi et al., 2011) / *Ai32* (*CAG-lox-stop-lox-ChR2*) (Madisen et al., 2012) on mixed genetic background were used for the optogenetic behavioral studies in Figure S3. Mice were singly housed during the period that experiments were performed. Mice were housed in plastic cages with disposable bedding on a 12 hour light/dark cycle with food available *ad libitum*, and water available *ad libitum* until experiments began, in which case they were placed on a water restricted regime as described below. Experiments were done during the dark phase.

METHOD DETAILS

Cortex-cerebellum connectivity tracing—Wild-type mice were injected with 500 nL *CAV-Cre* (Soudais et al., 2001) (genomic titers $\sim 10^{12}$ /mL) in one of several cerebellar folia (Vermis lobule VI: 750 μ m lateral of midline, 500 μ m anterior of the post-lambda suture; Vermis lobule VII: 750 μ m lateral of midline, between the mediolateral vessels separating lobules VI and VII, visible through the thin posterior skull surface; Simplex: 2.1 mm lateral, -5.9 mm from bregma; Crus2: 3 mm lateral, between the mediolateral vessels separating the crus1 and paramedian lobules, visible through the thin posterior skull surface). Mice were also injected with 500 nL of *AAV8-hSyn-FLEX-TVA-mCherry-2A-G* (genomic titer 10^{12} /mL) in the pontine nuclei bilaterally (-3.9 mm from bregma and 0.6 mm laterally). 2 weeks later, mice were injected in the pontine nuclei with G-deleted EnvA-pseudotyped Rabies-eGFP (genomic titers generally 10^9 /mL). The procedure follows previously established protocols (Schwarz et al., 2015).

Histology—We anesthetized mice using tribromethanol (Avertin) and transcardially perfused them with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). We extracted the brains into 4% PFA for 24 h of post-fixation, followed by at least 24 h in 30% sucrose solution. We cut 40 or 60 μ m tissue sections on a cryotome (Leica). Sections in Figure S1 were imaged using a slide scanner (Leica) and a 20 \times 0.8 NA objective.

For pontine rosette counting in cerebellar cortex (Figure S5O), we used a confocal microscope (Zeiss) with a 40 \times 1.4 NA objective to image 42 regions (area: 213 \times 213 μ m) from the dorsal surface of the cerebellum where we generally imaged *in vivo*, from 4 of the

mice that were used for optogenetic inhibition experiments. We collected a z-stack through the entire labeled thickness of the tissue (mean z-stack thickness: $28 \pm 1 \mu\text{m}$). We then visualized each z-stack as a volume in Imaris (Bitplane) and manually identified and counted mCherry-positive terminals in the volume.

Surgical procedures—Given the complexity of our multi-port optical preparation (Figure S2A) – consisting of a window over the cerebellum, prism in the motor cortex, bilateral fibers for pons inhibition, and two head-fixation headplates – we used computer-aided design (CAD) software (Creo Parametric, PTC) to plan our preparation. The model of the mouse skull was derived from micro-CT volumes (Mouse Imaging Centre, Toronto). In addition to ensuring the basic fit of all components on the mouse head, we used CAD planning to confirm that the microscope objectives used for the cortex and cerebellum did not collide with one another during imaging or with the implanted optical fibers.

We anesthetized mice using isoflurane (1.25–2.5% in 0.7–1.3 L/min of O_2) during surgeries. We removed hair from a ~10 mm diameter patch of skin over the skull, cleaned the skin with Betadine, and removed the patch of skin. We then peeled back connective tissue and muscle and dried the skull.

For cerebellar implants, we drilled a 3 mm diameter cranial window centered anteroposteriorly over the post-lambda suture and 1.5 mm right of the midline (Figure S2B, *left*). This positioned the window over cerebellar lobules VIa, VIb and simplex. To seal the skull opening, we affixed a #0 3 mm diameter glass cover slip (Warner Instruments) to the bottom of a 3 mm outer diameter, 2.7 mm inner diameter stainless steel tube (McMaster) cut to 1 mm height (Figure S2B, *top right*). We stereotaxically inserted the glass/tube assembly into the opening in the skull at a polar angle of 45° from the vertical axis and an azimuthal angle 25° from the midline (Figure S2B, *bottom right*). We then fixed the window in place and sealed it using Metabond (Parkell). We next affixed a custom stainless steel head fixation plate to the skull using Metabond and dental cement (Coltene/Whaledent). The 1.8 mm-thick headplate had a central 5 mm diameter opening to accommodate the glass/tube assembly, and two lateral extensions to permit fixing the plate to stainless steel holding bars during imaging and behavior. Among the cohort of mice, we took particular care to install the headplate at a consistent rotation angle about the implant axis, as variations in this angle could result in gross discrepancies in the positioning of the front part of the head containing the cortical implant.

For optogenetic inhibition of the pons, we first injected either *AAV8-hSyn-eNpHR3.0-mCherry* ($n=7$ mice) or *AAV8-hSyn-iC⁺⁺-mCherry* ($n=3$ mice) bilaterally into the basal pontine nuclei. We drilled two small holes (~0.5 mm) in the cranium 3.9 mm posterior to bregma and 0.6 mm left and right of the midline. We injected 500 nL of virus at a depth of 5.6 mm below lambda. We then implanted multimode fibers to illuminate the pons. Notably, the fiber implants (Doric Lenses, either 200 μm core/0.66 NA (3 mice) or 400 μm core/0.66 NA (7 mice)) had a short, 3.6 mm height ferrule (Figure S2C, *left*) which helped to avoid collisions with the imaging objectives. We stereotaxically inserted the fibers at a lateral tilt of 22° , entering the brain surface at 2.6 mm lateral of the midline, to a depth of 4.6 mm

along the insertion axis (Figure S2C, *right*). We cemented the fiber cannulas in place using Metabond.

For premotor cortical prism implants, we drilled a 3 mm diameter cranial window centered at the coordinates of the rostral forelimb area, 1.5 mm anterior of bregma and 1.1 mm lateral of the midline (Figure S2D). We then used a scalpel blade (EMS #11 feather) to make a 1.2 mm-long parasagittal incision centered in the opening (Figure S2E). After cleaning any bleeding, we used a fine forceps to carefully peel back the dura medial of the incision to facilitate insertion of the prism into cortex (Figure S2F). We found that leaving the dura intact over the tissue lateral to the incision (which is the tissue that is imaged by the prism) was critical for subsequent imaging quality. We glued a 1 mm right angle prism with protected aluminum mirror coating on the hypotenuse (Shanghai Optics) to the bottom of a 3 mm cover slip / steel tube assembly (Figure S2G). Using the stereotax, we inserted the prism into the incision at a tilt of 10°, lowering until the surface of the brain was maximally flush with the glass. We cemented the implant in place using Metabond.

For *Gad2-ChR2* optogenetic behavioral studies, we implanted the 3 mm window assemblies described above into the cerebellar area, as well as into the premotor area (but without a prism).

For orbitofrontal cortex implants (OFC), we drilled a ~2 mm diameter opening in the skull centered at 1.2 mm lateral of midline and 2.1 mm anterior to bregma. We removed the dura over the insertion area, then stereotaxically inserted a 1 mm diameter, 0.5 NA GRIN lens (GRINtech) glued to a 1 mm right angle prism mirror into the brain to a depth of 3.2 mm below bregma with the prism facing medially. We cemented the GRIN-prism probe in place using Metabond. As OFC columns are oriented vertically, our protocol kept the columns containing the imaged neurons intact. The OFC / cerebellum preparation (Figure S3L) provided greater clearance between objectives during simultaneous imaging than the premotor cortex / cerebellum preparation.

Two-photon microscopy—We performed all Ca²⁺ imaging using a custom two-photon microscope with two articulating objective arms (Lecoq et al., 2014). Each arm operated as an independent two-photon microscope (Figure S2H), with its own piezo-mounted (P-725.4CD, Physik Instrumente; or nPFocus100SL, nPoint Inc.) microscope objective and GaAsP photomultiplier tube (PMT; H10770PA-40, Hamamatsu). Each arm was also equipped with an “eyepiece” CMOS camera (DMK 23UV024, The Imaging Source) to visualize microscope positioning with bright-field illumination of the sample (with the main dichroic removed). We used a 40× 0.80 NA objective (LUMPlanFLN 40XW, Olympus) for all cerebellar imaging, covering a 250 × 250 μm field-of view (FOV); and a 20× 0.50 NA objective (UMPlanFLN 20XW, Olympus) for all cortical imaging. Premotor cortical imaging generally provided ~600 × 600 μm FOV, whereas OFC imaging through a 1 mm GRIN-prism probe (either #1050-002184, Inscopix; or custom-assembled) covered a 570 × 570 μm FOV.

A Ti:Sapphire laser (MaiTai, Spectra Physics) provided 920 nm excitation for two-photon imaging. For the cerebellum and premotor cortex, we used ~50–60 mW (each) after the

objective. For imaging the OFC, we used 80–90 mW after the objective, as measured above the implanted GRIN-prism probe.

Each microscope arm had six mechanical degrees-of-freedom (DOF): three translational DOFs to position the objective tip in space, two rotational DOFs to adjust the orientation of the optical axis, and fine, piezo-controlled movement along the objective axis. In addition, the sample stage had three translational DOFs, and the behavioral apparatus could be finely rotated (in the *xy* plane) on top of the sample stage. In total, our imaging apparatus afforded 16 mechanical degrees-of-freedom to achieve simultaneous imaging of cortex and cerebellum (Figure S2I).

To align the two microscope objectives to the cerebellar and cortical implants, we utilized the laser back-reflection technique described previously (Wagner et al., 2017), but in the following three-step order (Figure S2J). In Step 1, we aligned the 40× objective to the cerebellar window primarily using the “vertical” rotation of the objective (*i.e.*, rotation in the *yz* plane) and the translational DOFs and “horizontal” rotation (*i.e.*, in the *xy* plane) of the sample holder. Next, in Step 2, we aligned the 20× objective to the cortical implant utilizing only the translational and rotational DOFs of that objective. Finally, in Step 3, for fine tuning and live adjustment of the imaging FOVs, we utilized only the translational and piezo DOFs of each objective.

We used ScanImage 5.2 software (Vidrio Technologies) to control each microscope. All movies were acquired at 512 × 512 pixel resolution at ~30 Hz frame rate. For all except iC++ experiments, we operated the two microscopes asynchronously. The precise temporal relationship between the two Ca²⁺ movies was established by sampling both microscopes’ frame clocks with a common digitizer (Logic 8 Pro, Saleae). For iC++ experiments, we explicitly synchronized the two microscopes at the frame level by providing the frame clock of one microscope to the external trigger input of the other. We assessed the level of synchronicity by recording both pairs of frame and line clocks. We observed a slight, variable delay (~0.1 ms; to be compared with a frame period of ~33.3 ms) in the timing of the externally triggered frame relative to the “master” frame clock. The delay was taken explicitly into account in the iC++ deinterlacing procedure.

Image preprocessing and extraction of Ca²⁺ signals—We applied a common image preprocessing pipeline to all two-photon movies. First, we corrected for any DC offset in the pixel values (which can originate, for example, from an arbitrary bias in the PMT preamplifier). For each frame, we computed the minimum pixel value over the entire image. We then averaged this value over all frames, and subtracted the result from all pixels in the movie. Next, we corrected brain motion using piecewise rigid motion correction (NoRMCorre) (Pnevmatikakis and Giovannucci, 2017) over 64 × 64 pixel patches of the image. We then corrected for slow drifts in movie brightness over the course of the session (*e.g.* caused by slow loss of immersion fluid). We estimated a frame’s “brightness” by its mean pixel value over the entire image. We then fitted an exponential curve ($a * \exp(-b * t) + c$) to the brightness as a function of frame, and divided each frame by its fitted brightness. Finally, we z-scored each pixel using its mean value and standard deviation over all frames.

Two-photon movies acquired during simultaneous optogenetic perturbation underwent a modified version of the above pipeline. For iC++, we deinterlaced image frames during optogenetic perturbation periods (Figure S5J) prior to DC offset correction. For both iC++ and eNpHR3.0, we performed brightness drift correction by fitting only to the laser-off frames. We then computed the difference between average laser-on and laser-off images, to check for brightness increases during laser-on periods due to residual optogenetic light leakage. With eNpHR3.0, we typically observed a full-field brightness increase (~2%) on laser-on frames, whereas for iC++ we observed transient increases in brightness (~2%) following subfield transitions. We fitted these artifactual increases in brightness and compensated them on all laser-on frames.

To identify cells and extract their activity traces from the z-scored Ca^{2+} movies, we used the constrained non-negative matrix factorization (CNMF) cell-sorting algorithm (Pnevmatikakis et al., 2016), manually adding additional sources as necessary based on the neighboring-pixel correlation image. We utilized custom software written in MATLAB to visually check all candidate cells produced by CNMF and confirm that each had a morphology and Ca^{2+} activity trace consistent with an L5 cell or a GrC.

For downstream analyses, we did not use the deconvolved Ca^{2+} signals directly from CNMF, but recomputed the fluorescence traces by applying the manually confirmed spatial filters to the z-scored movie via least squares. We then removed high-frequency noise from these traces by low-pass filtering with a 2nd order Butterworth filter (−3 dB frequency at 4 Hz). We removed slow drifts from each trace by subtracting a 10th percentile-filtered (15 s sliding window) version of the signal.

Potentially inactive neurons—The CNMF algorithm only extracts neurons with detectable fluorescence activity. Other cells can be seen in background fluorescence that are not detected by CNMF. To obtain an estimate of the fraction of visibly GCaMP6f-expressing GrCs and L5 cells *without* detectable activity, and therefore *not* included in our analyses, we computed the mean projection image and manually counted the number of visible neurons ($n=4$ imaging sessions). We found that CNMF-extracted neurons were 45% of all visible GrCs, and 82% of total visible L5 cells. By applying ROIs to the manually-identified inactive neurons, we extracted fluorescence traces from these visible-but-not-CNMF-extracted cells. This analysis confirmed that “inactive” cells had few, if any, *bona fide* identifiable Ca^{2+} transients (Ca^{2+} event rate: 0.02 ± 0.002 Hz, mean \pm SEM, $n=459$ manually identified GrCs; 0.04 ± 0.004 Hz, $n=82$ L5 cells; compare to Figure S6H). The primary analyses that gave rise to our conclusions were derived from the structure of L5 and GrC *activity*: behavioral encoding (inactive cells neither contribute nor degrade population encoding); fractions of GrC activity explained by linear regression onto L5, or vice versa, and, similarly, best-match correlation coefficients (inactive cells are not recruited into regressors); dimensionality assessed by PCA (inactive cells do not contribute variance, and thus do not affect the dimensionality of activity). As a result, our primary conclusion—that the *activity* of L5 and GrC ensembles converges onto a shared, low-dimensional, task-encoding subspace of responses over learning—is unaffected by the inclusion or exclusion of silent cells. Interestingly, however, the greater number of inactive GrCs does suggest a

substantially larger “reservoir” of spare representational capacity in the GrC network, potentially engaged by contexts other than our behavioral task.

Relating GrC Ca²⁺ transients to spiking—Prior work imaging cerebellar granule cells with patch electrode recordings from the same cells *in vitro* estimated that 1 GrC action potential is likely associated with ~15% f/f GCaMP6f transients (Giovannucci et al., 2017). Similarly, *in vivo* anesthetized V1 recordings in L2/3 found 1 action potential was associated with ~15% f/f (Chen et al., 2013). To calibrate our Ca²⁺ data against these references, we converted our fluorescence traces into f/f units and then computed two critical quantities. First, we computed the *noise floor*, as the spread of the distribution of fluorescence values *excluding* Ca²⁺ transients. Across cells, we found that the 99th percentile value for the noise distribution was 16±0.2% f/f for GrCs and 12±0.2% f/f for L5 cells (mean±SEM across 568 GrCs and 368 L5 cells). Hence, it is likely that only multi-spike events consistently rose above our noise floor. Second, we examined the amplitudes of Ca²⁺ transient events used in analyses in Figure 3. We found that the mean Ca²⁺ event height was 48±1% for GrCs and 38±1% for L5 in f/f units (mean across cells), suggesting that most detected Ca²⁺ events fall into the multi-spike regime.

Behavior—For all behavior, mice were water restricted to 1 mL of water per day. Mice were monitored daily for signs of distress, coat quality, eye closing, hunching, or lethargy to assure general health. During behavioral training and imaging, mice generally received all water during daily training sessions. During all experiments, we recorded licking at 200 Hz using a capacitive sensor coupled to the metal water port, which delivered ~6 μ L 4% sucrose water reward near the animal’s mouth per successful trial. For all experiments, mice were head-fixed and their bodies were loosely constrained by a custom 3D printed transparent plastic tube.

The apparatus used for all forelimb tasks was as described previously (Wagner et al., 2017). In brief, 3D-printed plastic pieces were assembled into the configuration shown in Figure 1. The two primary linkages consisted of a passive “elbow joint” that rotated via ball bearings, and a “shoulder joint” actuated by a DC motor (Maxon DCX22) for which the rotation was measured by an optical encoder (Gurley Precision Instruments R120). A passive “wrist” joint connected the two linkages via a ball bearing at the handle. Control of the device was programmed in Labview (National Instruments) via a compact RIO chassis (cRIO 9024 with two 9505 motor driver modules and a 9403 digital I/O module) that communicated with a Windows PC. Software consisted of nested control loops: a 10 kHz FPGA controlled motor current and encoder reading; a 1 kHz real-time PC performed all other computations, including geometric transformations and force production calculations, and processes including data sampling, buffering and transfer to the Windows PC; and the Windows PC controlled high-level behavioral transitions including trial type specification, trial start and end, reward delivery, and data logging.

Training on the motor planning task proceeded in stages. Mice were first trained to push the handle forward in a virtual linear track by 7–8 mm to receive a sucrose water reward. Following movement, a 1 s delay preceded reward delivery, followed by another ~2 s delay before the robot automatically returned the handle to the mouse for the next trial, a process

which took ~1.5 s. Mice trained in this condition for 3–7 days, until they reliably performed ~200 trials in a 20–30-minute session.

After mice became proficient on linear movements, we exposed them to the movement sequence task, designed to assess motor planning similar to a previous study in humans (Sheahan et al., 2016). Initially, mice moved in a loose right angle track with a 6 mm forward segment followed by a 6 mm lateral segment, directed either left or right. The loose track was implemented by a proportional-integral-derivative (PID) controller that responded to increasing deviation of the handle from the desired trajectory with an increasing opposing force. Specifically, during the first segment until forward motion reached 6 mm, the robot opposed only lateral motion. During the second segment, the robot only opposed forward/backward motion. Deviations less than 0.05 mm were ignored by the controller. The parameters of the control loop did not perfectly cancel sudden forces exerted by the mouse. Thus, mice had some ability to veer off-track (typically up to ~1 mm, Figure 1D, 7E). Left and right movements alternated in blocks of 40 successful trials. In this phase, if mice pushed the wrong way (*i.e.*, left during a right trial or vice versa) and collided with the virtual walls of the track, they were permitted to recover by pushing in the correct direction. When mice were proficient at this task (minimum 3 days), we changed the task contingency such that if they pushed in the wrong lateral direction during the lateral segment of the trial beyond a threshold (either 0.5 mm or 3 mm), the robot locked in place, ending the trial without reward. The 3 mm threshold drove learning more effectively in mice poor at the task. Following the standard delay (2 s), the handle automatically returned to the mouse for the next trial. We found that this final contingency was critical for mice to pay close attention to their intended turn directions. Highly trained mice could reliably perform ~160–240 trials (*i.e.*, 4–6 blocks of 40) in a 20–30-minute session. For chronic imaging, Day 1 refers to the first day mice were exposed to the loose right-angle track.

Cortical and cerebellar optogenetics studies—*Gad2-Cre/Ai32* mice implanted with windows over both the premotor cortical and cerebellar regions that we imaged throughout the paper were trained on the task. During optogenetic manipulation sessions, two collimated optical fibers (~3–4 mm beam diameter) were positioned ~1 cm above each of the two windows to deliver 445 nm light (OBIS 445, Coherent). The laser was pulsed at 50 Hz with 10 ms pulse duration. Time-averaged optical powers were 1–5 mW for the cerebellar window and 9–25 mW for the cortical window, distributed over the ~3 mm of tissue exposed in the windows. These power levels were chosen based on tests during the forward-only movement task. The lower cerebellar power was necessary to avoid inducing right forelimb tremor which precluded forelimb movements required by our task. We trained the mice for 1–2 weeks on the movement sequence task without optogenetic perturbation. During perturbation experiments, following baseline performance (~1–5 minutes), one of the two fibers was activated for 1 minute. After the fiber was turned off, mice were given 1–5 minutes to recover their prior task performance (~10–20 successfully executed trials) before the next 1-minute laser-on period began. Mice received 1–2 laser-on periods for each brain region and for each movement direction (left or right) per experiment. Each of the 3 mice underwent 3 experiments.

Pontine optogenetics studies—We performed pontine inhibition experiments in conjunction with single-site cerebellar imaging in 4 mice and with dual-site premotor and cerebellar imaging in 6 mice. For pontine manipulation during cerebellar imaging without cortical imaging, we mounted the fibers from the laser (FG025LJA, Thorlabs) directly on-axis with the implanted fibers (Figure S5A and S5B) using standard ferrule mating sleeves (Doric Lenses). For pontine inhibition during dual-site imaging, it was necessary to re-route the optogenetic fibers to avoid the cortical objective. We designed a custom micro-optical assembly, consisting of a GRIN lens collimator and a right angle prism mirror, to fold the fiber axis by 90° above the implanted fibers (Figure S5C). While the GRIN-prism component (#1050-002186, Inscopix) was not originally designed for this application, optical simulation (Zemax) showed that it sufficed to mode-match the output light of the incoming fiber onto the face of the implanted fiber (Figure S5D). Using the fold adapter on both fiber implants allowed for optogenetic manipulation of the pons during dual-site imaging without collisions (Figure S5E).

To actuate eNpHR3.0 (Figure S5F), we utilized a 594 nm laser (OBIS 594, Coherent) filtered by a 592/8 bandpass filter (FF01-592/8-25, Semrock). During pons inhibition trials, we opened a shutter to allow for continuous-wave illumination of the pons. In the emission path of the two-photon microscopes, we inserted a 594 nm notch filter (NF03-594E-25) to suppress excess photons from leaking into the PMT. For eNpHR3.0, spectral separation was sufficient to allow for GCaMP6f imaging in the cerebellum and dorsal cortex despite concurrent 594 nm illumination in the pons (Figure S5H).

To actuate iC++ (Figure S5G), we utilized a 488 nm laser (LuxX 488-200, Omicron), filtered by a 482/18 bandpass filter (FF02-482/18-25), and equipped the two-photon microscopes with a 496 nm longpass filter (FF01-496/LP-25). In contrast to the eNpHR3.0 / 594 nm case, spectral separation did *not* allow two-photon imaging of GCaMP6f during concurrent iC++ actuation (Figure S5I). Hence, to allow for simultaneous iC++ actuation during two-photon imaging, we devised a temporal multiplexing protocol in addition to spectral separation.

Our temporal multiplexing scheme utilized a duty ratio of ~1:1 between iC++ actuation and GCaMP6f imaging, thereby reducing the effective imaging frame rate to $30/2=15$ Hz. During optogenetic perturbation, we divided the imaging frame into an odd number (*e.g.* N=11) of subfields. On odd-numbered imaging frames, we imaged the odd subfields (Figure S5J, *top left*) and enabled the 488 nm laser during even subfields; and on even-numbered frames we imaged the even subfields (*top middle*) and enabled the 488 nm laser during odd subfields. Hence, every pair of optogenetics-interlaced frames could be combined to produce a full frame image (*top right*). We implemented the multiplexing scheme with a microcontroller (Arduino Mega 2560) that took as inputs an “opto enable” TTL signal along with the frame and line clocks from the microscope and computed a “laser enable” TTL signal that modulated the 488 nm laser (Figure S5J, bottom).

During optogenetic imaging experiments, we activated the laser on 20% of trials for the entire duration of the trial (*i.e.*, from the return of the handle from the previous trial, until the return of the handle for the subsequent trial, overall ~4 s).

Chronic imaging studies—For the chronic studies in Figures 5–7, imaging began from the first day on the movement sequence task. We imaged each mouse multiple days over the first 7–9 days of training on the task, by which point they had achieved general competency. We called any sessions acquired during the first 3 days “early” learning, and afterwards “mid” learning. For all mid-learning sessions error turns were penalized by aborting the trial with no reward. After this initial imaging period, we continued training mice for another 7–14 days *without* imaging until they achieved asymptotic task performance. We then returned for final imaging sessions (“late” learning). We performed these experiments in 7 mice, but we were only able to track individual neurons from early to late learning in 4 of those 7. Thus, all analyses requiring consistent cell identity were restricted to the 4 mice in which we successfully tracked neuronal identity over learning.

Aligning cells across days—First, we performed image registration – either MATLAB’s built-in intensity-based affine registration (*imregtform*), control point-based projective geometric mapping (*fitgeotrans*), or rigid-only NoRMCorre registration – to rigidly align each day’s mean image to that of every other day. This step primarily accounted for lateral variations in the field-of-views on each day. We supplemented the rigid alignment with NoRMCorre’s non-rigid registration to correct for any nonlinear discrepancies in the mean images. We thus obtained spatial transformations between every pair of days, which allowed spatial filters from each day to be “imported” into the coordinate frame of every other day.

Next, we sought to maximize the number of matching cells across days. Generally, cells absent one day but detected on another day could be due to multiple reasons: (1) the cell was not present in the movie due to lateral or axial shifts in the imaging field; (2) the cell was present in the movie, but not active; or (3) the cell was both present and active but simply “missed” by the extraction algorithm. Because it was not always possible to distinguish between (1) and (2), we maximized the number of cross-day matches only by minimizing the number of cells falling into case (3) for each day.

To identify potential “missed” cells, we used previously computed spatial transformations to determine which cells were identified on one day but missing on another (“unmatched” cells). We then “imported” the unmatched spatial filters. For a particular day, this produced multiple sets of possibly missed cells, derived from each of the other days. We then extracted traces for all filters while eliminating duplicates, and manually examined each one to determine whether a cell was in fact present with at least one Ca^{2+} fluorescence transient. For ~50% of such missed cell candidates, transients were in fact present. By including “missed” cells in this way, the number of cells on each day typically increased by ~2 \times .

Finally, using the “missing cell”-corrected datasets, we performed cell map alignments between the last training day to all other days. Across datasets, $35\pm 3\%$ of cells present on the last day were present on all previously imaged days.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavior analysis—For trial-locked and trial-averaged analyses, we used either the onset of lateral motion or the time of reward delivery to align trials. We defined the turn onset as

the time when lateral motion crossed a 0.5 mm threshold. In cases where mice made small, back-and-forth lateral movements, we used the final threshold crossing as the midpoint. We divided trials into two main categories. “Pure” left or right turning trials were those in which the animal’s lateral motion did not stray more than 0.5 mm in the wrong direction. “Error” trials were those in which mice attempted to move in the wrong direction.

For analyses of task encoding, to minimize behavioral differences between mice and between training days, we restricted analysis to pure left or right turns which were more kinematically similar. For all other analyses including correlations and dimensionality assessment we included all trials.

Analysis of neural task encoding—We performed two main analyses of cells’ task-encoding. The first was a single-cell analysis (Figure 2E,F 5G,H,K) in which we used linear regression to “reproduce” a cell’s single-trial fluorescence activity from a set of behavioral regressors. We defined the set of behavioral regressors as indicator signals active over one of the following time windows: the 300 ms before (“pre-turn”) or after (“post-turn”) turn onset, the 300 ms before (“pre-reward”) or after (“post-reward”) reward delivery. We further segregated these signals by left and right turn trials. For each GrC or L5 cell, we then linearly regressed its activity traces during all pure left and right turn trials onto these 8 behavioral indicators. Cells with a significant regression coefficient for *either* turn direction were considered “modulated” in that corresponding time window. If a cell additionally had a significantly greater coefficient for one direction than for the other then it was considered “direction-preferring” in that window. We also tabulated the variance in each cell’s activity explained by this regression (Figure 5I).

To determine significance for the single-cell behavioral regression analysis, we performed two permutation tests. The first permutation test determined significant modulation. We generated randomized data sets where the times of each trial’s turn onset (the alignment point) was chosen randomly from the full recording. We then aligned all fluorescence activity to these random “trials,” and performed linear regression using the true regressors. If the true weight given to a regressor was greater than that given in 95% of the shuffles, the cell was deemed to be “modulated” by that regressor. The second permutation test determined significant direction preference. We generated randomized data sets by randomly permuting the left/right trial labels of the true fluorescence activity. We then recomputed the same regressions using the randomized left/right fluorescence activity for each shuffle. If the true difference between the weight assigned to a left regressor and that assigned to the corresponding right regressor was larger than the corresponding difference between those regressors in 95% of the shuffles, the regressors were deemed to be significantly different. If, in addition, the preferred regressor had also been deemed to significantly *modulate* the cell by the previous permutation test, the cell was deemed to be significantly direction preferring in the corresponding time window (pre/post turn or reward).

The second analysis of task encoding employed ensemble activity. In this analysis, we used linear regression to reproduce behavioral signals from the single-trial activity of all cells. The behavioral signals were: a “movement” signal (–300 ms to + 300 ms relative to turn onset) and a “reward” signal (–200 ms to +400 ms relative to reward delivery) for each turn

direction, as illustrated in the top row of Figure S6A. We then performed a separate linear regression for each of these “behavioral signals,” using the fluorescence activity of all cells on all trials. An example output of this regression is shown in (Figure S6A). We then tabulated the variance in behavioral signals explained by this regression (Figure 5J, K and Figure 7B, C; for each session, averaged across the regressions for each of the 4 behavioral signals).

Dimensionality analysis—Dimensionality analysis was performed using PCA across cells—i.e., to identify the main contributors to variability across cells (as opposed to variability across trials). We performed two types of analysis. The first analysis was dimensionality of *single-trial* activity (Figure 2I and 5H, I). In this case, we performed PCA across cells. In the data matrix, each column was the fluorescence of one cell concatenated across all trials (pure turns, errors, aborted trials). Thus, the data matrix was of size (T×N)-by-C, where T is the number of trials, N is the number of timepoints per trial (from -2 to 2 s relative to turn onset), and C is the number of neurons. We then plotted the fraction of total ensemble L5 or GrC signal variance as a function of the number of principal components included in the reconstruction of the original population activity.

The second analysis was dimensionality of *trial-averaged* activity (Figure S6B; “signal” dimensionality, analogous to “signal” correlations described below). In this case, we computed the trial-averaged activity of every cell on successful left and right turn trials separately and then concatenated the left- and right-trial averages and recorded the results in a data matrix in which each column was the trial-averaged activity of one cell. Thus, the resulting data matrix was of size 2N-by-C. We then performed PCA across cells.

Correlations and L5-GrC regressions—We characterized every GrC by its correlations both to all other GrCs, as well as its correlations to all L5 neurons. Thus, each GrC was associated with two distributions: a distribution of GrC correlations, and a distribution of L5 correlations (full distributions shown in Figure S3I). For each GrC, we summarized these distributions by the best-match correlation, i.e., the maximum of the distribution (Figure 3B). We then tabulated this statistic for all neurons, yielding the distribution of best-match GrC-GrC and L5-GrC correlations. The motivation for using the best-match correlation was two-fold: first, this allowed us to compare representations in similar L5-GrC pairs (e.g., Figure 3); second, the distributions were very heavy-tailed, and the maximum captured the changes in this tail. Nevertheless, as shown in Figure S3I, the full distributions conveyed qualitatively similar information. For each L5 neuron we similarly computed the correlations with all other L5 neurons, and we recorded the best-match correlation for every cell, yielding the distribution of L5-L5 best-match correlations.

To compute correlations in trial-averaged signals (often called “signal correlations”, Figure S6F) or in trial-to-trial variability (often called “noise correlations”, Figure S3K and S6G), we followed standard techniques (Cohen and Kohn, 2011). For correlations in trial-averaged responses, we computed the trial-averaged response of each cell (averaged for left and right turns separately) and then correlated the trial-averaged responses of different cells (with left and right responses concatenated). For correlations in trial-to-trial variability, we concatenated all the single-trial activity for each cell, subtracted from each single trial

fluorescence trace the trial-averaged response (for left and right turn trials separately), and then computed the correlations between the mean-subtracted concatenated single-trial responses.

We used linear regression to determine the variance of each individual GrC's single-trial activity that was explained by the L5 ensemble as an alternative to pairwise correlations (Figure 6C and 7D). We linearly regressed each GrC's single-trial fluorescence traces concatenated across trials onto all the corresponding traces of all L5 cells. We recorded the R^2 fraction of variance explained by this regression. We also performed regression using the trial-averaged activity (Figure 2L). In this case, we computed the trial-averaged activity of every cell on successful left and right turn trials separately and then concatenated the left- and right-trial averages and recorded the results in a data matrix in which each column was the trial-averaged activity of one cell. Thus, the resulting data matrix used for regression was of size $2N$ -by- C .

To regress the entire GrC ensemble single-trial activity onto the entire L5 ensemble activity and also determine the minimum dimensionality of L5 activity needed to best explain GrC activity, we performed Reduced Rank Regression on the concatenated single-trial activity of all GrCs and L5 cells and tabulated the variance explained and the rank of the regression (Figure 6D).

Due to the marginally different L5 vs GrC sampling rates (variable, but typically ~ 30.03 Hz in GrC and 29.97 Hz in cortex), we linearly interpolated the L5 signals to match the GrC sampling rate when directly matching time points. In addition, GrC ensembles were slightly larger on average than L5 ensembles (86 ± 7 vs 73 ± 7 cells). To ensure that our results were not impacted by this discrepancy, we performed a Monte Carlo subsampling analysis in which we repeatedly randomly sampled a subset of whichever of the two populations was larger (L5 or GrC), to be the same size as the smaller population, prior to recomputing best-match correlations (as in Figure 3C) or regressions (as in Figure 2H), and produced results nearly identical to those reported in the Figures (data not shown).

Ca²⁺ event-based analysis—For Ca²⁺ event-based analyses (Figure 3), we performed threshold-based peak detection (MATLAB's *findpeaks* function) using a 1.5 SD threshold and requiring a minimum event separation of 500 ms (motivated by GCaMP6f Ca²⁺ kinetics). For event matching between one GrC and one L5 cell, we tabulated any events occurring within 300 ms of one another as “matched.” Matching was only performed on highly correlated cells ($r > 0.4$). While we believe that these parameters are appropriate given the temporal characteristics of Ca²⁺ imaging data, we also confirmed that the principal conclusion drawn from these data—that there are more GrC-only events than L5-only events, for correlated pairs—did not depend on parameters. Namely, we repeated the analysis of Figure 3F using more fine-grained parameters: 250 ms event bins and 150 ms event-matching separation. Under these conditions GrC-only events continued to outnumber L5-only events ($p < 10^{-6}$ Wilcoxon sign rank test). More precise estimates than this are impeded by the Ca²⁺ indicator kinetics.

We computed the Kullback-Leibler (KL) divergence (Figure 3I) to compare the temporal distribution of shared L5-GrC events aligned to the task to that of GrC-only events. We computed time histograms for GrC-only and shared L5-GrC events (−2.5 s to 2.5 s with 0.5 s time bins) separately for left and right turn trials (and concatenated the histograms for the two directions). We collapsed all events that were outside this interval with respect to movement into the ends of the histogram. We then computed the KL divergence as

$$-\sum_{\text{time bins}} P_{\text{shared}} \log \frac{P_{\text{GC-only}}}{P_{\text{shared}}}.$$

Optogenetic GrC response analysis—To determine which GrCs were inhibited or disinhibited by optogenetic inhibition of pontine neurons (Figure 4, Figure S5), we employed a permutation test. For each GrC, we computed the trial-averaged response on left and right trials separately, and on laser-off and laser-on trials separately. We found the timepoint with the largest *decrease* in trial-averaged activity on laser-on trials compared to laser-off trials (between −2 s and 2 s), t_{max} . We then averaged the decrease in an 800 ms window centered on t_{max} (magnitudes shown in Figure 4E). We then computed the same quantity for trial-shuffled data, in which, for every shuffle, we randomly permuted the laser-off/laser-on trial labels (but only permuted among trials of the same turn direction). If the true maximum decrease was greater than that observed on 99% of the shuffles and if the maximum decrease was at least 0.5 z-scores of fluorescence, we tabulated the cell as significantly inhibited by optogenetic inhibition of pontine neurons. We similarly determined whether cells were significantly disinhibited.

Clustering changes in correlations—To identify clusters of L5 cells and GrCs for which correlations evolved coherently during learning, we first computed the correlation coefficients between all possible pairings among the set of L5 cells and GrCs tracked every day throughout learning (4 mice). To identify clustered *changes*, we computed the difference between the correlation coefficient matrix on the final day of imaging and on the first day of imaging. We performed *k*-means clustering on the correlation coefficient difference matrix (“kmeans” function in MATLAB, *k*=2, although conclusions were unchanged when using three or four clusters). To compute the normalized mutual information between cell type and cluster membership, we used the formula: $NMI(W, C) = \frac{I(W; C)}{(H(W) + H(C)) / 2}$ where the mutual

$$\text{information } I(W; C) = \sum_k \sum_j \frac{|w_k \cap c_j|}{N} \log_2 \frac{N |w_k \cap c_j|}{|w_k| |c_j|} \text{ and the entro } H(W) = \sum_k \frac{|w_k|}{N} \log_2 \frac{|w_k|}{N},$$

in which *N* is the total number of neurons, $|w_k|$ is the number of neurons in cluster *k*, and $|c_j|$ is the number of L5 cells or GrCs, following (Schütze et al., 2008). The normalized mutual information represents the reduction in uncertainty about cell type provided by knowledge of the cluster assignment, relative to the total uncertainty in cell type and cluster assignment measured by entropy, and thus varies from 0 to 1.

Statistics—We used MATLAB (Mathworks) for all statistical tests. We compared medians of two groups using the Wilcoxon rank-sum test. We probed the median difference between groups of paired samples using the Wilcoxon signed-rank test. We also compared the median of a distribution to zero using the Wilcoxon signed-rank test. These nonparametric tests do not assume the data follow a particular statistical distribution. Histogram error bars

were computed from counting statistics as $\sqrt{N\left(1 - \frac{N}{N_{total}}\right)}$, where N =number per bin and N_{total} =total elements.

In some cases where standard statistical tests could not be applied, we used custom permutation tests described in the corresponding analysis sections.

For all statistical tests and all data presentations in each main and supplemental Figure, the n value used to evaluate significance or generate the data figure is indicated in each Figure legend or in each citation in the Results text, as appropriate.

Simulations (Figure S4)—Simulated GrCs. Similar to prior work, we implemented a simulated granule cell input circuit containing N^{GrC} GrCs. Each GrC, σ_i , $i = \{1, \dots, N^{GrC}\}$ had binary output (active/inactive) for simplicity. Binary neurons, as previously published (Billings et al., 2014; Gilmer and Person, 2017; Litwin-Kumar et al., 2017), are a reasonable simplification since GrCs have very low tonic firing rates with bursty responses to mossy fiber input (MF) (Chabrol et al., 2015; Chadderton et al., 2004). Simulated GrCs received exactly four MFs. Feedforward inhibition modeled gain control effects of Golgi cells (Billings et al., 2014) by dynamically setting GrC firing thresholds, as originally posited by Marr-Albus (Albus, 1971; Marr, 1969). The dynamic threshold allowed activation of only the top $f^{GrC}=10\%$ of GrCs ranked by input strength at each time point (Babadi and Sompolinsky, 2014; Litwin-Kumar et al., 2017). GrCs activity levels were taken from the data (binarized: mean probability of a Ca^{2+} event in each GrC in each 500 ms time bin was 0.1).

We had no way to accurately determine the fraction of MFs arising from task-related L5 signals. To very roughly estimate, we considered that our tracing studies showed that premotor cortex contributes 15% of cortex-via-pons inputs to this region of the cerebellum (Figure S1). Although the pontine nuclei are the largest source of MFs (Sillitoe et al., 2012), without knowing the true fraction of MFs contributed by pons, we considered a scenario where pons contributed around half of all MF inputs. Thus if each GrC receives input from four nearby MFs at random, combinatorics implies that each GrC has probability $p_{input}=0.27$ to receive at least one MF of premotor origin, of which only a small probability (3%) comes from receiving two or more of these inputs which, for simplicity, we ignored in this simulation. An important feature of our imaging data is that 55% of GCaMP-labeled GrCs had few detectable fluorescence transients (see Methods section above). This suggests that most of these GrCs are very unlikely to receive task-related input, and thus suggests that cells from which we extract activity are substantially *more* likely to receive task-related input. To represent this unknown but likely substantially higher effective probability of receiving task related MF input, we varied p_{input} widely between 0.4, 0.5, and 0.6, which yielded qualitatively similar results. For simplicity we therefore display only $p_{input}=0.5$. (In additional simulations not shown, we explored the possibility that MFs cluster in individual GrCs, such that 50% of premotor-recipient neurons received more than one premotor-derived MF. We explored whether the standard MF integration model could recapitulate our data under these conditions, but due to the heterogeneity of selectivity

within premotor L5 itself, this results in GrCs that mix away the selectivity in L5, thereby failing to match our data).

Each MF contributed to the postsynaptic membrane potential of recipient GrCs via synaptic weights drawn from a Gaussian distribution. Due to the dynamic threshold set by feedforward inhibition, and the use of binary GrCs, there is a free choice of scale, thus the magnitude of the moments of the distribution are arbitrary. Formally, the activity of each GrC can be written as

$$\sigma_i^{GC} = \Theta \left(\sum_{i=1}^{N^{MF}} w_{ij} \sigma^{MF} - T \right).$$

Where Θ is the Heaviside step function which is equal to one in its argument is positive and is zero otherwise. Each row w_i of the connectivity matrix had exactly 4 non-zero entries, one of which with probability p_{input} arose from the pontine MF population. The dynamic threshold, representing feedforward inhibition from Golgi cells, was then set to ensure that $\sum_i^{N^{GC}} \sigma_i = f^{GC}$.

Simulated MFs: MF activity was also assumed to be binary, with the probability of activation for each MF in each time bin parametrized by f^{MF} . The constraints provided by the observed GrC activity levels in our data (described above), and satisfying the assumption that GrCs generally require two or more active MFs to fire (Chadderton et al., 2004), were sufficient to determine $f^{MF}=0.15$. Alternatively, when we considered a model where GrCs can fire with only 1 active MF (Figure S4D,E), we thus required that $f^{MF}=0.1$.

We simulated two MF populations: “task-related MFs” originating from the pontine layer receiving input from the task-related L5 pool; and a pool of “all other MFs.”

Since for the “all other MFs” population we had no information about response properties or potential correlations to the “task-related MF” pool, we parametrized the correlation among all mossy fibers (x -axis in all panels similar to Figure S4B). We implemented this by generating the “all other MFs” activity as a random projection of the activity of the task-related MF pool combined with noise. At one extreme parameter value, each MF in the “all other MFs” pool reproduced one task-related MF exactly, while at the other extreme, the activity of the “all other MFs” pool was purely random. This maintained the same MF activity level at all correlation parameter values.

For the “task-related MFs,” we simulated a pontine layer. Each pontine cell received input from the L5 layer. Like the GrCs, each pontine cell’s output was binary and in the active state when the weighted sum of its inputs exceeded threshold:

$$\sigma_i^{MF-L5} = \Theta \left(\sum_{i=1}^{N^{L5}} J_{ij} \sigma^{L5} - T \right).$$

Here N^{L5} is the number of pons-projecting L5 neurons. The threshold in this layer was chosen to produce a pontine activity level of f^{MF} .

There were two possible mechanisms for the high selectivity (i.e., pre/post left/right movement/reward) of GrCs in our imaging data, which was in turn similar to what we observed in L5: (1) Selectivity was inherited from L5 cells, which would also require pontine cells to be selective. (2) GrCs generate selectivity *de novo*, which would require GrCs to integrate multiple MF inputs that carry similar weakly selective information. As random combinatorial inputs to GrCs makes (2) very difficult (Litwin-Kumar et al., 2017), we therefore assumed that pontine activity is selective. Therefore, to preserve selectivity in pontine output, we set the synaptic weights from L5 to pons via a simple Hebbian association rule. Specifically, each pontine neuron had a “desired” output activity pattern over the set of stimuli, denoted by x_{μ}^{pons} , an N^{pons} -dimensional binary vector indicating which pontine neurons are responsive to stimulus μ . The Hebbian association rule ensures selective responses similar to the desired activity patterns (Babadi and Sompolinsky, 2014) by setting the synaptic weights to

$$J_{ij} = \frac{1}{N^{L5}} \sum_{\mu}^P x_{i,\mu}^{L5} x_{j,\mu}^{pons},$$

Where P is the number of stimuli, x_{μ}^{L5} with $\mu = 1, \dots, P$ is the N^{L5} -dimensional vector indicating which L5 neurons are responsive to stimulus μ . Since some L5-pons convergence exists (Brodal and Bjaalie, 1997), but the precise convergence ratio is unknown, we set N^{L5} to be 5 times larger than the pontine layer. Varying this ratio from 1.25, to 2.5, to 5 produced very similar results (data not shown).

Simulated L5 cells.: Input to the model was a set of canonical stimuli, numbering $P=10$. Each stimulus activated a set of L5 cells, with each L5 cell responding to each stimulus with probability $f^{L5} = 0.1$ (again based on event rates in the data). In addition, noise parametrized by η corrupted each L5 cell response, so that each L5 cell had probability $f^{L5} \eta$ to fire for a stimulus to which it was not responsive, and probability $(1 - f^{L5}) \eta$ to *not* fire to a stimulus to which it was responsive. This noise structure produced L5 activity levels that were constant across noise levels. The noise level η allowed control of L5-L5 correlations, and was thus chosen to match correlations to the data (when binarized into events with 500 ms bins, described in Methods section above).

Dominant MF model.: Under an alternative model, task-related MFs (e.g. from premotor L5 via pons) become substantially stronger than other MFs, such that they ‘dominate’ the GrC’s output. We simulated dominant MFs by setting their synaptic strength sufficiently high to activate the recipient GrC with high probability, i.e. synaptic weights above the average GrC activation threshold of 2MF in the random mossy fiber integration model.

Correlation quantification.: In all simulation data panels in Figure S4, correlations were quantified, for each neuron, by the *spread* of its distribution of correlation coefficients to

other neurons (standard deviation). Thus to compare to the data, data correlations in this case were computed as SD of the distribution of correlation coefficients across cell pairs, rather than best-match correlations. This was necessary, because the number of neurons simulated (10,000 GrCs) was much greater than the number recorded in an imaging session, precluding a direct comparison of best-match correlations.

Simulated GrC selectivity.: To measure the selectivity of each of the GrC neurons to the different P stimuli (e.g., Figure S4C), we calculated a response vector for each GrC, measuring the fraction of trials on which it responded to the noisy MF input resulting from that stimulus. Each GrC i thus had a P -dimensional vector with values λ_{μ}^i , $\mu = 1, \dots, P$, each between zero and one. We then calculated the dispersion of these values using

$$d_i = 1 - \frac{\left(\sum_{\mu}^P \lambda_{\mu}^i\right)^2}{P \sum_{\mu}^P \left(\lambda_{\mu}^i\right)^2}$$

Where the dimension ratio $d_i = 0$ implies the GrC responded equally to all patterns, and at the upper bound $d_i = 1 - 1/P$ implies the neuron was selective to a single stimulus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- First simultaneous recordings from neocortex and cerebellum over weeks of learning
- Cortical layer 5 and cerebellar granule cells show similar task encoding in experts
- Learning increases correlations among initially dissimilar L5 and granule cells
- L5 and granule cells converge to similar, low-dimensional, task-encoding activity

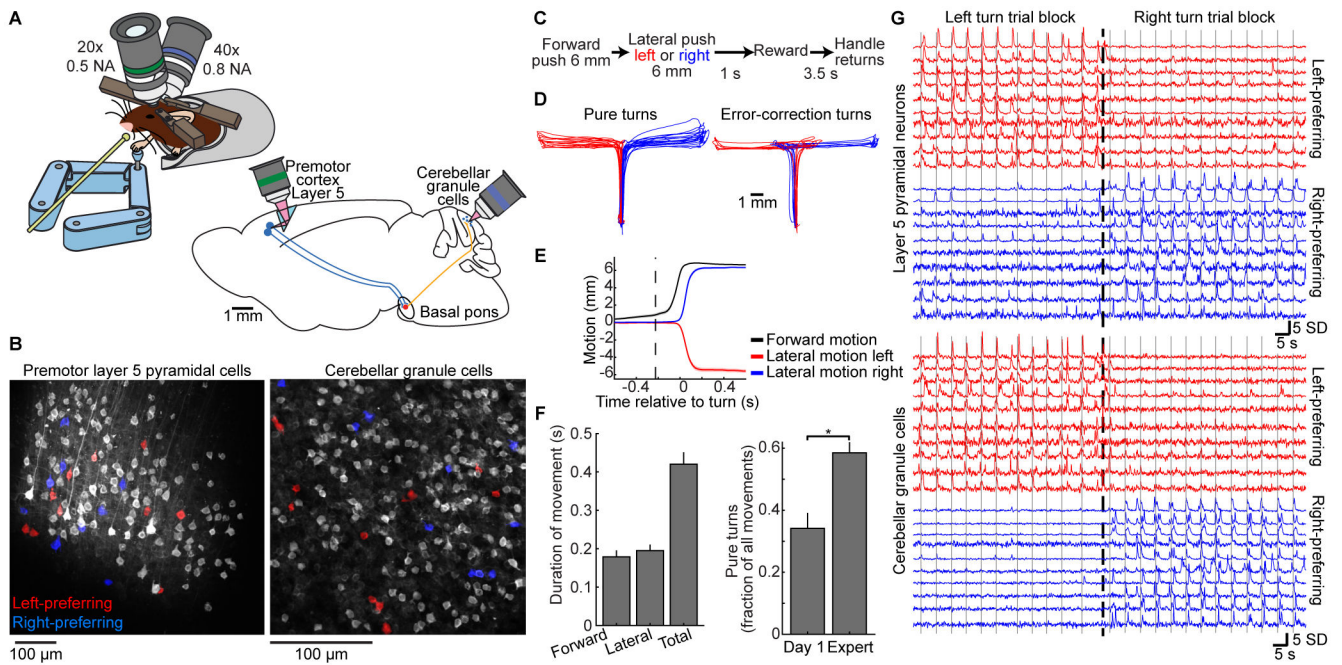


Figure 1. Simultaneous Two-photon Ca^{2+} Imaging of Cerebellar GrCs and Premotor Cortex L5 Pyramidal Neurons during a Forelimb Movement Task

(A) Experimental schematics. Mice voluntarily moved a manipulandum for sucrose water reward (left). We performed simultaneous Ca^{2+} imaging in cerebellar GrCs through a cranial window, and in L5 pyramidal neurons of the premotor cortex using an implanted 1 mm prism (right). GCaMP6f was expressed in L5 cells and GrCs using quadruple transgenic mice *Rbp4-Cre/Math1-Cre/Ai93/ztTA*.

(B) Mean images from representative two-photon Ca^{2+} imaging movies in L5 cells (left) and GrCs (right). The spatial filters used to extract fluorescence traces from cells with detected activity are highlighted in grayscale or red/blue (see G below; $n=144$ L5 cells/177 GrCs).

(C) Forelimb movement task. Water-restricted mice self-initiated trials. The task alternated blocks of 40 forward/left-turn movements with blocks of 40 forward/right-turn movements. No cues indicated trial type.

(D) Example movements on the virtual right-angle track (left panel, $n=20$ each of pure left and right turns; right panel, $n=8$ error-correction turns in each direction).

(E) Average motion over time in forward (black curve) and lateral (colored curves) directions for all pure turn trials in the session in D, aligned temporally to turn onset ($n=51/63$ pure-left/pure-right turns). Dashed vertical line denotes average forward movement onset.

(F) Behavioral performance: left, average duration of forward and turning portions of pure turn trials ($n=28$ imaging sessions in 10 expert mice). Right, pure turns are more common after learning ($p=0.003$, Wilcoxon rank sum test, $n=7/21$ for Day-1/Expert sessions in 7 mice).

(G) For the imaging session in B, example fluorescence traces from both cortex (top) and cerebellum (bottom). SD, standard deviation (fluorescence in z-scored units). Dashed vertical line indicates time of switch from a left-turn block to a right-turn block of trials. Solid vertical lines denote individual turn motions. Traces show direction-preferring cells

colored by their direction preference ($n=20$ example L5 cells and GrCs, 10 preferring each direction; 11 turn motions in each direction). Corresponding cell spatial filters are colored in **B**.

See Figures S1–S3 for related anatomy, methods, and necessity of imaged areas for behavior.

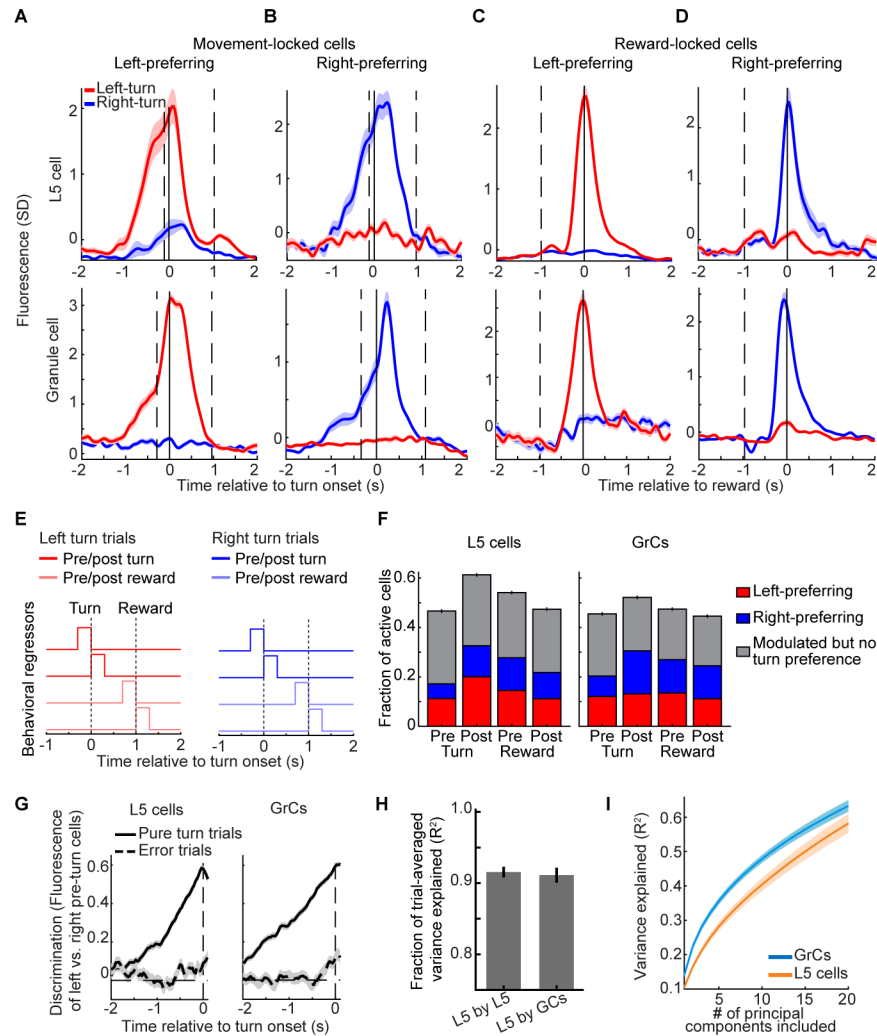


Figure 2. Similar Task Representations in L5 Cells and GrCs in Expert Mice

(A–D) Trial-averaged activity in example L5 cells (top) or GrCs (bottom) that responded preferentially before and during either left- or right-turn movements (A and B), or reward consumption (C and D) following either successful left- or right-turn trials. Vertical lines from left to right in A and B denote average forward motion onset, turning motion onset, and average reward delivery time. Vertical lines in C and D denote average time of turning motion and reward delivery. Shaded areas denote SEM in this and all subsequent figures. (From left to right for L5 cells: 109/76/72/109 left- and 74/71/71/74 right-turn trials; for GrCs: $n=68/97/72/109$ left- and $69/99/71/74$ right-turn trials).

(E) Individual neurons were scored by linearly regressing their concatenated single-trial activity onto a set of 8 behavioral regressors.

(F) Fraction of cells with significant coefficients for either turn direction (grey) or with significantly larger coefficients for one turn direction (colored) among L5 cells (left) and GrCs (right) ($n=2,037/2417$ for L5 cells/GrCs from 28 imaging sessions in 10 mice; these and all subsequent histogram error bars are from counting statistics).

(G) All GrCs or L5 cells that responded preferentially prior to one turn direction were grouped. The discrimination index for each trial was the time-varying difference between the average activity of left- and right-preferring pre-turn cell groups. Traces show average discrimination index across all pure-turn or all error trials (sign of index was inverted on right trials to match sign of left trials; $n=1,498$ pure-turn trials and 612 error trials, on which incorrect lateral motion exceeded 2.5 mm, from 5 mice. Index normalized to range from -1 to 1). On error trials, neither ensemble discriminated turn direction prior to lateral motion onset (from -300 to -50 ms relative to turn onset; $p=0.22/0.67$ for 720/536 GrCs/L5 cells with pre-turn direction preference, Wilcoxon signed rank test).

(H) Time-varying trial-averaged activity of each L5 neuron was reproduced by linear regression from the activity of either L5 or GrC populations. Regressions performed at similarly high levels (R^2 , fraction of variance explained on held-out data; 28 sessions in 10 mice).

(I) PCA was performed across cells, using the fluorescence concatenated across all trials of each individual L5 cell or GrC. Fewer principal components are needed to explain 50% of GrC variance than are needed for L5 ($p=0.002$, Wilcoxon sign-rank test, $n=28$ imaging sessions from 10 mice).

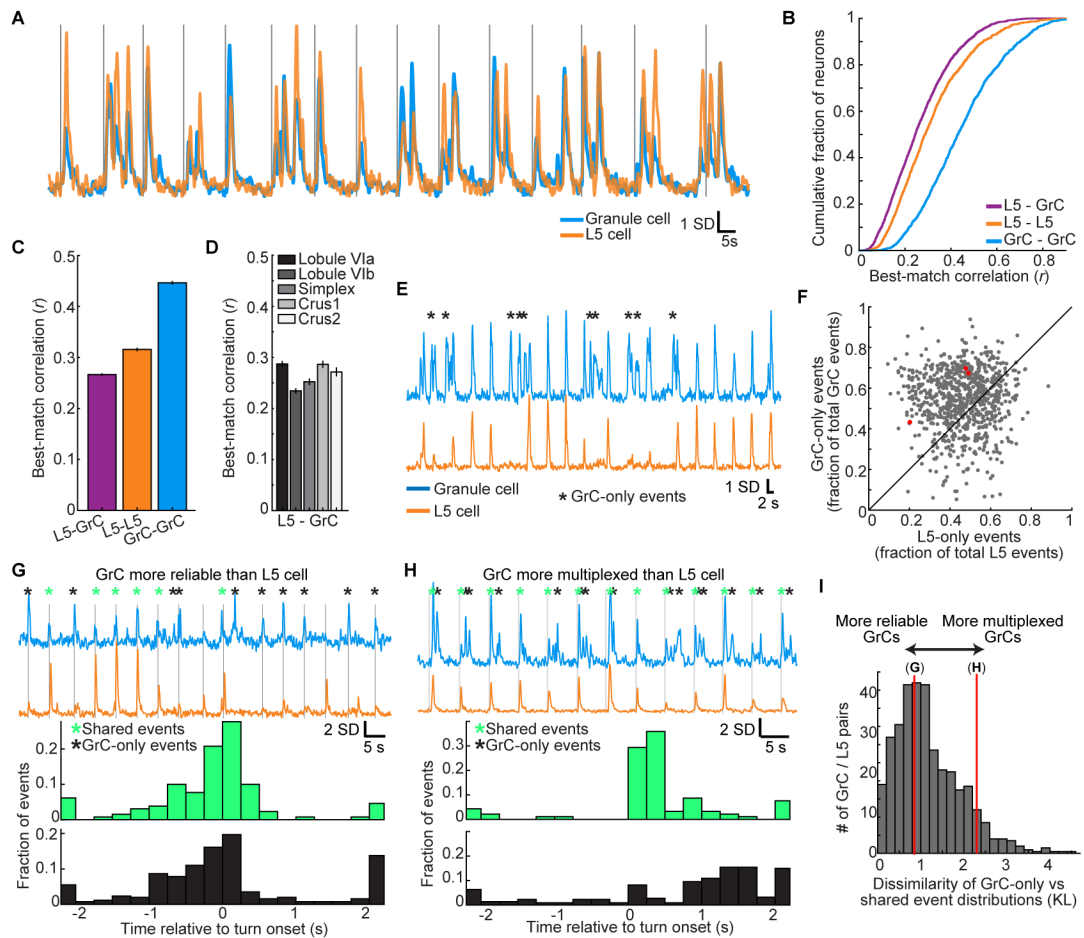


Figure 3. Highly Correlated Single-trial L5-GrC Activity in Expert Mice

(A) Example of a highly correlated L5 cell-GrC pair. Vertical lines denote individual turning motion onsets.

(B and C) Cumulative distributions of correlation coefficients between each GrC or L5 cell and its best-matching GrC or L5 cell (mean \pm SEM; $n=2,037/2,417$ L5 cells/GrCs; computed over the concatenated activity on all movements from -2 to 2 s relative to turn onset).

(D) Correlations with imaging sessions grouped by the cerebellar lobule that was imaged.

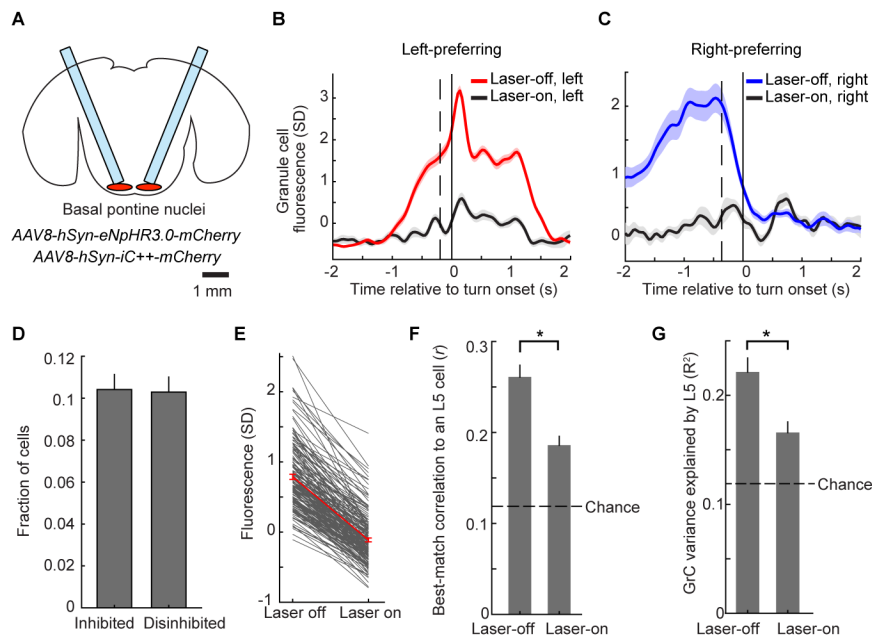
(E) Example highly correlated L5-GrC pair ($r=0.59$). Black asterisks indicate GrC events not present in the L5 cell.

(F) Scatter plot of all highly correlated L5-GrC pairs (defined as $r>0.4$; each dot is a pair) showing the proportion of total L5 events that were unique to the L5 cell (x -axis), compared to the proportion of GrC events that were unique to the GrC (y -axis). GrC-only events were substantially more common ($p<10^{-6}$ Wilcoxon signed rank test, $n=800$ L5-GrC pairs with $r>0.4$ from 28 imaging sessions in 10 mice). Red dots indicate examples from E, G, and H, from left to right.

(G) Top, fluorescence traces from a highly correlated L5-GrC pair ($r=0.43$), with onset of individual turn motions denoted by vertical lines. Asterisks denote L5-GrC shared events (green) or GrC-only events (black). Bottom, the temporal distribution (relative to forelimb movement) of shared events is very similar to the temporal distribution of GrC-only events.

(H) Same as **G**, for a L5-GrC pair ($r=0.41$) in which the temporal distribution of GrC-only events strongly diverged from that of L5-GrC shared events.

(I) Histogram of the dissimilarity (Kullback-Leibler divergence, KL, STAR Methods) between the temporal distribution of shared events and the distribution of GrC-only events, for all highly correlated pairs. Red vertical lines indicate example pairs in **G** and **H** with KL divergences of 0.85 and 2.3, respectively. Most cell pairs are more similar to **G** than to **H**. See Figure S3 for additional data analyses, and Figure S4 for theoretical analyses.



See Figure S5 for methods and related data.

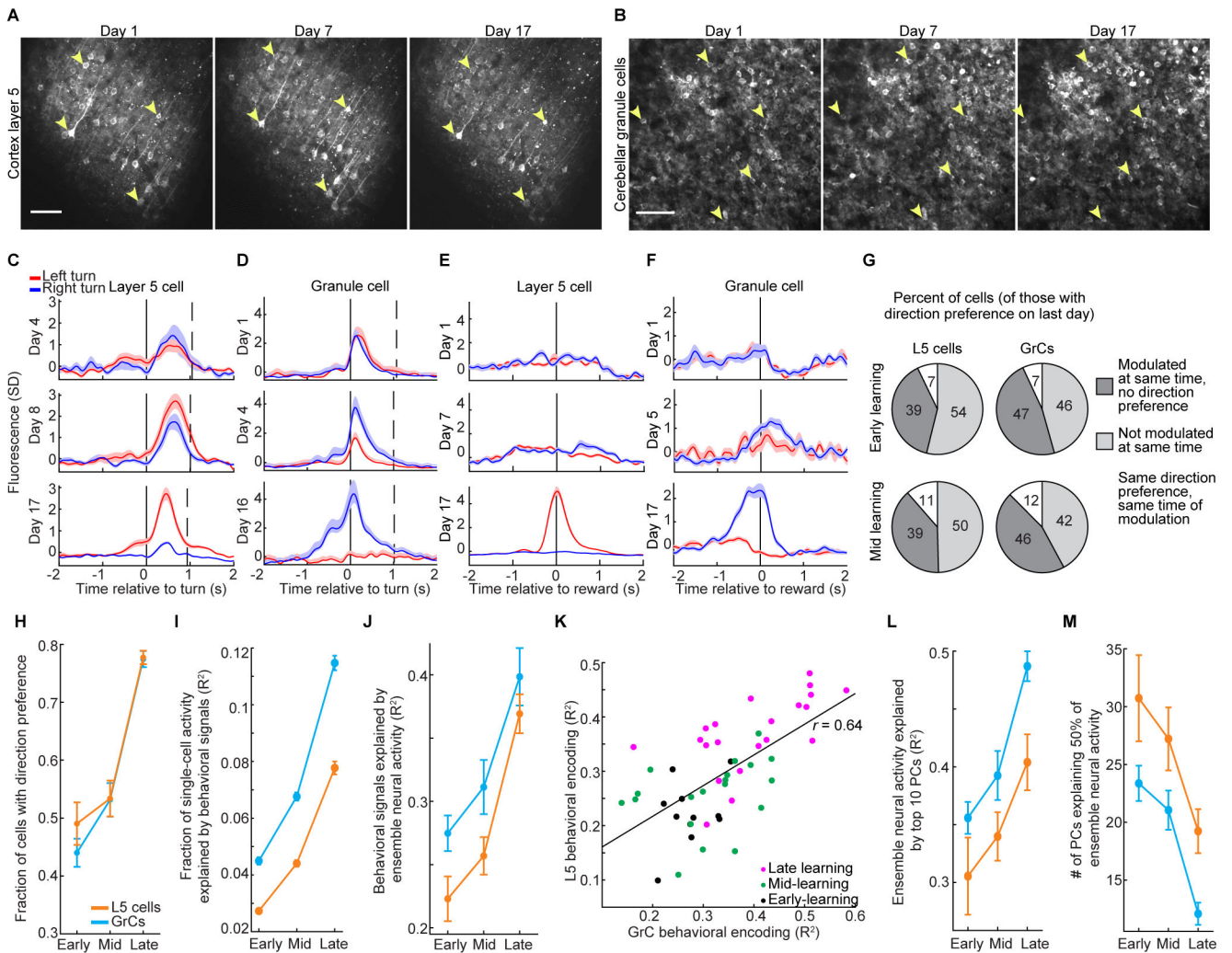


Figure 5. Common L5 and GrC Task Representations Emerge Concurrently during Learning

(A and B) Example mean fluorescence images of the same L5 cells (A) and GrCs (B) acquired over learning. Arrowheads point to example cells that were tracked across days. (C–F) Trial-averaged activity of example L5 cells (C and E) and GrCs (D and F) shown on days corresponding to early, mid, and late learning. Cells develop direction-prefering activity time-locked to movement (C and D) or reward (E and F) (mean±SEM; left/right turn trial numbers for C, D, E, F: Early: $n=40/18$, 24/68, 29/18, 29/18; Mid: 34/27, 57/33, 48/36, 17/45; Late: 109/74, 24/37, 72/71, 72/71).

(G) All cells were scored on each day for direction preference and task-locking using regression analysis as in Figure 2E. For the set of all L5 cells (left) and GrCs (right) that had direction preference on the final day of imaging, activity was primarily either modulated at the same time but without direction preference (dark gray) or was not modulated at that time (light gray) on earlier days ($n=183/206$ L5 cells and 172/202 GrCs from early/mid-learning, respectively). Direction-prefering activity was only infrequently maintained (white).

(H and I) Based on regression analysis (as in Figure 2E), more cells had direction-preference late in learning (H, $p=6\times 10^{-6}$ and 5×10^{-6} for early vs. late for L5 and GrCs;

Wilcoxon rank sum test; $n=11$ early, 19 mid, and 21 late imaging sessions from 7 mice), and regressions more accurately reproduced each cell's activity (**I**; $p < 10^{-6}$ Wilcoxon rank sum test for early vs late in L5 and GrCs; $n=1,265/1,397, 2,113/2,324, 1,666/1,647$ L5/GrC observations early, mid, and late, respectively).

(**J** and **K**) The entire ensemble of GrCs or L5 cells was scored for its fidelity of behavioral encoding. The accuracy of reproducing behavioral signals shown in Figure S6A via single-trial linear regression rose over learning (**J**, mean \pm SEM; late vs early, $p=0.0009$ and $p=9\times 10^{-5}$ for L5 and GrC respectively; $n=11$ early, 19 mid, and 21 late learning imaging sessions from 7 mice). In addition, regression accuracy for GrC populations (x -axis) and L5 populations (y axis) in each imaging session (colored dots) covaried over learning (**K**, 51 imaging sessions from 7 mice).

(**L** and **M**) L5 and GrC ensembles both became lower-dimensional over learning, as the top 10 principal components (as computed in Figure 2I) explained greater fractions of single-trial variance (**L**, $p=5\times 10^{-6}$ and 3×10^{-5} for GrCs and L5 cells, respectively), and fewer components were required to explain 50% of variance (**M**, $p=4\times 10^{-5}$ and $p=0.007$ for GrCs and L5 cells respectively, Wilcoxon rank sum test, 11 early, 21 late sessions). See Figure S6 for further analyses and related data.

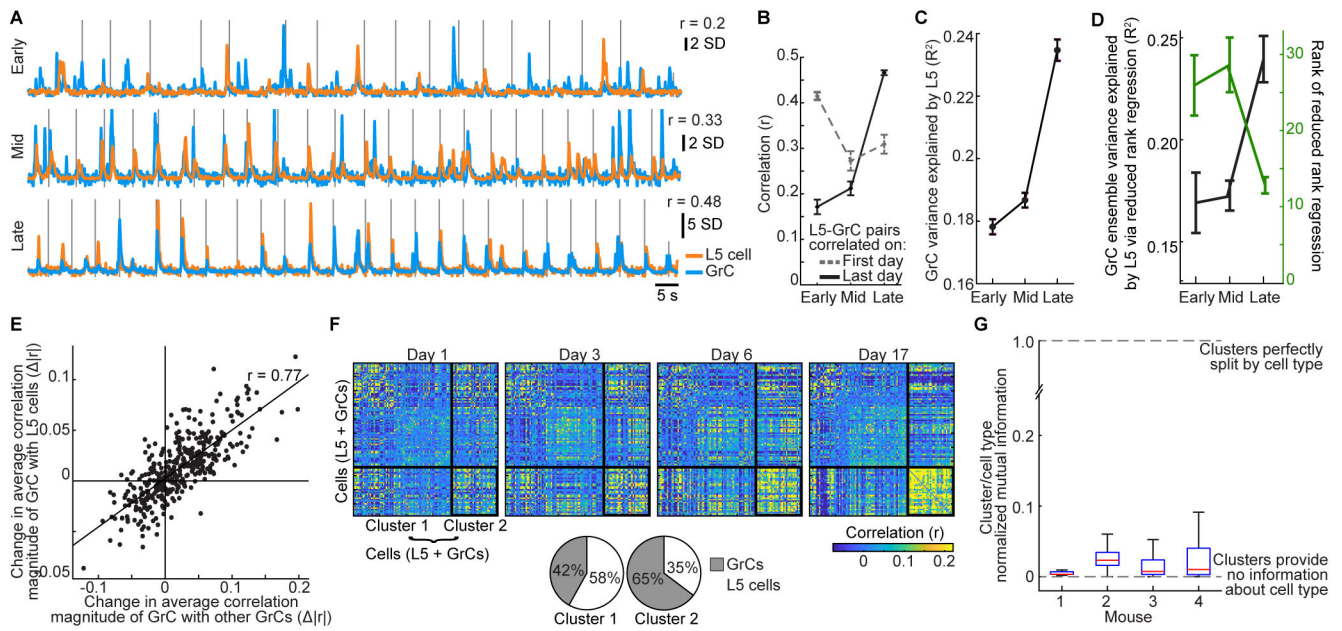


Figure 6. Shared Cortico-cerebellar Dynamics Emerge Over Learning

(A) Example L5-GrC pair strongly correlated late in learning was poorly correlated early in learning. Vertical lines denote onset of individual turn motions.

(B) L5-GrC pairs that were highly correlated on the last day were weakly correlated early in learning (solid trace; $p < 10^{-6}$ Wilcoxon signed-rank test, $n = 121$ pairs with last-day correlations > 0.4). Dashed trace shows the evolution of correlations for pairs with high correlations early in learning. The initial correlation for last-day-correlated pairs was weaker than the final correlation of first-day-correlated pairs ($p = 3 \times 10^{-6}$ Wilcoxon rank sum test, $n = 61$ pairs early learning correlations > 0.4).

(C) The accuracy with which L5 population activity reproduced the fluorescence of each GrC via linear regression rose over learning (curves show mean \pm SEM across GrCs; $p < 10^{-6}$ comparing early and late learning, Wilcoxon rank sum test).

(D) The single-trial activity of *all* GrCs was simultaneously reproduced via linear reduced rank regression using L5 population activity. Regression accuracy rose over learning (black), while the average rank (dimensionality) of the L5-GrC regression fell (green; curves show mean \pm SEM across imaging sessions from 7 mice).

(E) For each GrC (represented by a dot), the change in its average correlation magnitude to all other GrCs (*x*-axis) strongly covaried with the change in its correlation to all L5 cells ($n = 398$ GrCs tracked over learning).

(F) Matrix of correlation coefficients between each pair of neurons for 4 days between early and late learning in one mouse ($n = 55/53$ L5 cells/GrCs). *K*-means clustering ($k = 2$) identified groups of neurons that together exhibited similar changes in correlation to all other neurons over learning. Clustering was applied to the *differences* in correlation coefficients between Day 17 and Day 1. The thick solid black outlines in the matrix show the resulting clusters. The neurons are sorted in the same order on each day. Bottom, pie charts show substantial contribution of GrCs and L5 cells to both clusters.

(G) The correspondence between cluster membership and L5/GrC cell type was characterized via the normalized mutual information. Mutual information was generally close to zero, indicating that L5 cells and GrCs were recruited together into coherently evolving cell assemblies during learning. Boxes show median, 25/75th percentiles over 1,000 clustering instantiations.

See Figure S6 for further analyses and related data.

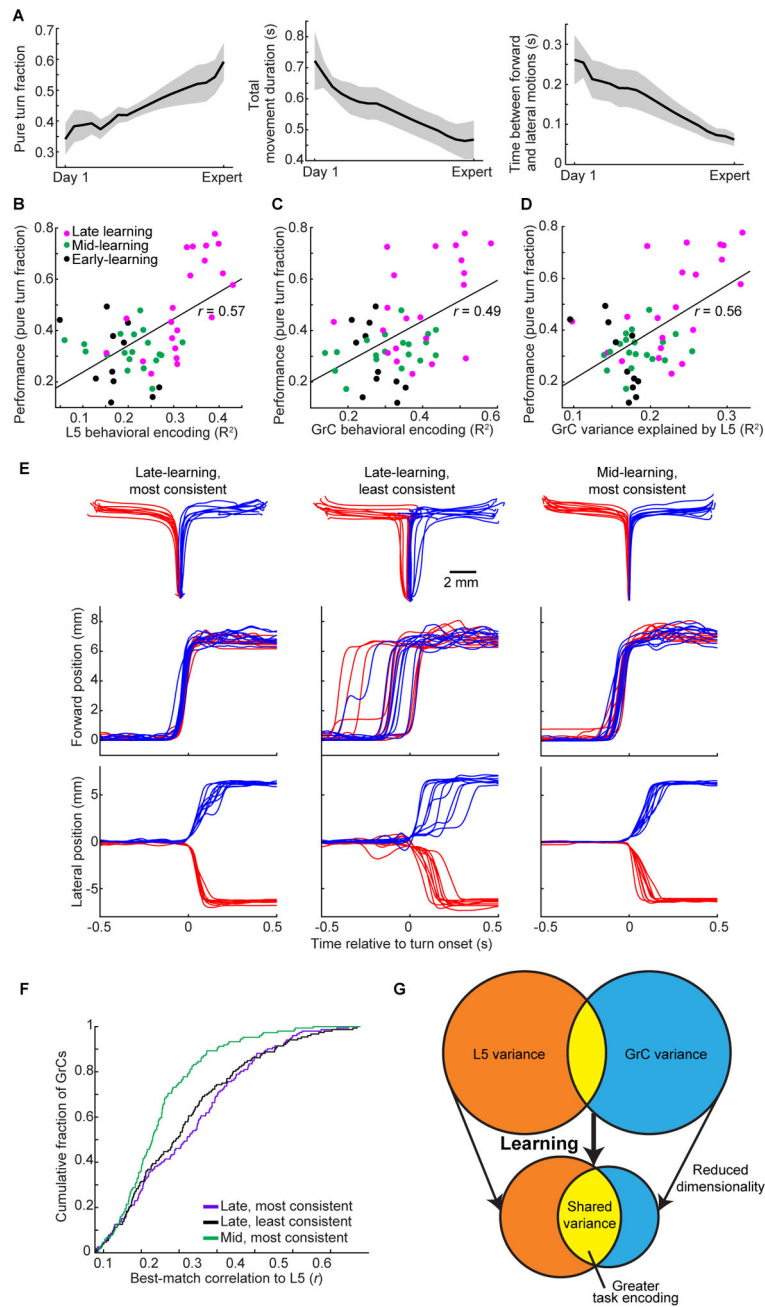


Figure 7. Coherent L5-GrC Dynamics Reflect a Learned Circuit State.

(A) Behavioral learning. Pure turns as a fraction of trials increased (left, $p=0.003$, $n=7$ mice). Total movement duration decreased (middle, $p<10^{-6}$), reflecting briefer transitions between the forward and lateral motions (right, $p<10^{-6}$; $n=460/2,062$ Day-1/late learning trials; 19.3 ± 1.1 days between day 1 and last day). Statistics compare all Day 1 to all late-learning days, using trials from all mice (2.8 ± 0.5 expert days per mouse; Wilcoxon rank sum test). (B, C) Behavioral encoding via linear regression in L5 (B) and GrC (C) ensembles covaried with behavioral performance over learning.

(D) The fraction of GrC variance explained by L5 ensembles via linear regression also covaried with behavioral performance.

(E) Within each imaging session, each successful trial was ranked by its kinematic similarity to the average pure turn trajectory of the same direction. From an example mouse, 10 trajectories in each direction are shown from three sets of trials: from a late-learning day, the subsets of trials most and least consistent with the average trajectory (left two columns), and from a mid-learning day, the subset of trials most consistent with the average trajectory (right column). Top row shows trajectories in x - y space, and middle and bottom rows show forward and lateral motion over time.

(F) For each late- and mid-learning imaging session, best-match L5-GrC correlations were computed using only trials from either the most consistent or least consistent subset (20 top- and bottom-ranked trials in each direction). L5-GrC correlations were not significantly different between most and least consistent trials on the late learning day (distributions shown for mouse in **E**; black versus grey, $p=0.35$, Kolmogorov-Smirnov test, $n=149/152$ GrCs, and 143/134 L5 cells, from the mid-/late-learning days, respectively). By contrast, even the *most* consistent trials on the *mid*-learning day exhibited substantially smaller L5-GrC correlations than did the *least* consistent *late* learning trials ($p=0.0001$).

(G) Schematic of evolution of L5 and GrC ensemble dynamics over learning. From an initially less coherent, higher-dimensional, less task-related set of activity patterns, L5 and GrC ensembles converge onto a more shared, low-dimensional, task-encoding set of activity patterns.

See Figure S7 for further analyses and related data.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Virus strains		
AAV8-hSyn-FLEX-TVA-mCherry-2A-G	UNC vector core	N/A
EnvA-Rabies- G-GFP	Salk vector core	Stock name: G-deleted Rabies-eGFP
AAV8-hSyn-eNpHR3.0-mCherry	Stanford vector core	Stock# GVVC-AAV-147
AAV8-hSyn-iC++-mCherry	Stanford vector core	Stock# GVVC-AAV-162
CAV-Cre	Soudais 2001	N/A
Chemicals		
Isoflurane	Henry Schein Animal Health	CAS# 26675-46-7; CHEBI:6015
C&B Metabond Quick Adhesive Cement System	Parkell	UN/ID# UN1247
Avertin (2,2,2-Tribromoethanol)	Sigma	CAS# 75-80-9; SKU# T48402
DAPI	Thermo Fisher Scientific	Cat# D1306
Experimental Models: Organisms/Strains		
Mouse: Ai93(TITL-GCaMP6f)-D	Jackson Labs	Stock# 024103
Mouse: zfTA	Jackson Labs	Stock# 012266
Mouse: Rbp4-KL100	GENSAT	Founder# KL100
Mouse: Math1-Cre	Jackson Labs	Stock# 011104
Mouse: GAD2-IRES-Cre	Jackson Labs	Stock# 010802
Mouse: Ai32 (LSL-ChR2-EYFP)	Jackson Labs	Stock# 012569
Software and Algorithms		
MATLAB	Mathworks	https://www.mathworks.com
IMARIS	Bitplane	https://www.bitplane.com
CNMF	Simons Foundation/Flatiron institute; Pnevmatikakis 2016	https://github.com/flatironinstitute/CaImAn-MATLAB
NoRMCorre	Simons Foundation/Flatiron institute; Pnevmatikakis 2017	https://github.com/flatironinstitute/NoRMCorre
ScanImage	Vidrio Technologies	http://scanimage.vidriotechnologies.com/
LabVIEW	National Instruments	http://www.ni.com/en-us/shop/labview.html