Cortico-cerebellar coordination facilitates neuroprosthetic learning

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Abstract

Temporal coordination among neurons and development of functional neuronal assemblies is central to nervous system function and purposeful behavior. Still, there is a paucity of evidence about how functional coordination emerges in neuronal assemblies in cortical and subcortical regions that are directly related to the control of functional output. We investigated emergent neural dynamics between primary motor cortex (M1) and the contralateral cerebellar cortex as rats learned a neuroprosthetic/ brain-machine interface (BMI) task. BMIs offer a powerful tool to causally test how distributed neural networks achieve specific neural patterns. During neuroprosthetic learning, actuator movements are causally linked to primary motor cortex (M1) neurons, i.e., “direct” neurons, that project to the decoder and whose firing is required to successfully perform the task. However, it is unknown how such direct M1 activity interacts with cerebellar activity. We observed a striking 3-6 Hz coherence that emerged between these regions’ local-field potentials (LFPs) with neuroprosthetic learning which also modulated task-related spiking. We found a robust task-related indirect modulation in the cerebellum, and we found that it became synchronous with M1-direct activity with learning. We also performed optogenetic inhibition of cerebellar activity and found that this led to performance impairments in neuroprosthetic control. Together, these results demonstrate that coordinated neural dynamics emerge in cortico-cerebellar regions during neuroprosthetic learning which supports task-relevant activity in M1 direct neuronal populations, and further, that cerebellar influence is necessary for M1-driven rapid direct neural control.

Keywords: Cerebellum, motor cortex, brain-machine interface
Introduction

To accomplish even the simplest of tasks, the nervous system coordinates activity across distant brain regions. For example, holding a bottle full of water requires several neurons to produce well-calibrated muscle forces for grasping and monitoring sensory feedback. Parallel processes in several sensorimotor regions underlie even the most trivial tasks such as above. Dense reciprocal connectivity between these regions supports this processing which likely need to be configured rapidly and flexibly to support a repertoire of behavior and afford learning of new skills. Two motor regions, the primary motor cortex (M1) and the cerebellum, have dense reciprocal connections, and are known to be involved in motor learning\(^1,2\). Studies have shown how learning alters local activity in the cerebellum or M1\(^3–6\), yet learning also alters task-related cross area coordination\(^2,7\). Both M1 and the cerebellum have dense connections with other cortical and subcortical regions, and hence it is difficult to ascertain if changes in interaction are because of direct interactions between M1 and cerebellum\(^8–10\), or because of their roles coordinating the upper limb.

To isolate the effects of M1 and cerebellum to movement control versus the direct task-related coordination, we have used a brain-machine interface task, where select M1 neurons (‘direct’ neurons) modulate their activity to control an external disembodied actuator. This offers experimenters a powerful paradigm where they can dictate/set the neuron-behavior relationship. During “brain control”, direct M1 neurons change their firing properties during neuromuscular prosthetic learning\(^11–18\). In addition, other neurons in the local M1 network also become task-coupled (i.e., ‘indirect’ neurons)\(^12,13,15,19–23\). It remains
unknown what activity emerges in the cerebellum with M1-driven neuroprosthetic learning and what role it plays. Here, we hypothesized that cerebellum neurons will demonstrate task-related firing during M1-driven neuroprosthetic learning. We also predicted that modulating the cerebellum optogenetically will impact M1-driven neuroprosthetic learning.

Another focus of our investigations was on co-emergent synchronous activity across M1 and cerebellum with neuroprosthetic learning. Recent theories have proposed that alterations in the pattern of synchronous activity across regions can serve to coordinate network activity for natural and neuroprosthetic behaviors\textsuperscript{20,24}. Such transient local-field potential (LFP) activity can modulate excitability of cell groups across varying spatiotemporal scales\textsuperscript{25,26}. This helps in achieving precise temporal control in neural networks that can enhance information transfer in specific cell populations\textsuperscript{27} and can influence spike-timing-dependent plasticity\textsuperscript{28}. Such temporally coordinated activity among ensembles underlies diverse neural processes ranging from perception, decision making, action, memory and attention\textsuperscript{6,29–33}. Importantly, spiking in one region becomes coordinated with LFP in another region, indicative of synchrony\textsuperscript{30,34}. Despite the evidence that synchronous LFP is related to learning\textsuperscript{6,35}, there is a paucity of evidence that this selectively modulates task-relevant activity of neurons across brain areas. Using the neuroprosthetic task paradigm, where we can control the neurons that are linked to behavioral output, we aimed to disentangle the synchronous activity of “direct” neurons within M1 locally and with task-relevant indirect activity in the cerebellum to understand how these diverse classes of cells were modulated by synchronous LFP.
We have used a BMI paradigm in which a small set of M1-direct neurons controlled a simple 1-D actuator\textsuperscript{12,18,21}. We recorded additional neural activity from neighboring indirect neurons in M1, as well as distant cerebellar cortex. First, we found that cerebellar neurons developed strong 'task-related' indirect modulation, and M1 and cerebellum developed a 3-6Hz LFP coherence with neuroprosthetic learning. We also found that direct M1 neurons and task-related indirect neurons in both M1 and cerebellum enhanced their phase-locking to this 3-6Hz oscillation in the LFPs. Next, we also found that synchronous spiking between M1 direct neurons and cerebellar indirect neurons increased with neuroprosthetic learning, and fine timescale coordination between these regions also increased as evaluated through canonical correlation analyses. In our last set of experiments, we optogenetically inhibited the cerebellum and found that this led to performance impairments in the neuroprosthetic task and weakening of M1-direct activity. Together, our results support a necessary role of cross-area interactions between M1 and cerebellum that underlie neuroprosthetic skill learning.
Results

We implanted microwire electrode arrays in the primary motor cortex (M1) and silicon probes in a tetrode/polytrode configuration in the cerebellar posterior lobes (see Methods for details). After neural implant surgery, we trained 8 animals to exert direct neural control on the angular velocity of a mechanical actuator that can also deliver water. A linear decoder with randomized weights typically converted the firing rates of two groups of units in M1 (randomly selected and assigned positive or negative weights; hereafter referred to as ‘direct’ units; M1 TRd’s) into the angular velocity of the actuator. We also recorded multiple other units in both M1 and cerebellum that were not causally linked to actuator movements but showed task-related modulation (hereafter referred to as ‘indirect’ units). The decoder weights were held constant during the session to exclusively rely on neural learning mechanisms. Each trial started with the simultaneous delivery of an auditory tone and opening of a door to allow access to the tube (Fig. 1 a,b). At the start of each trial, the angular position of the tube was set to resting position, P1. If the angular position of the tube was held for >400 ms at target position P2, a defined amount of water was delivered (that is, successful trial). A trial was stopped if this was not achieved within 15s (that is, unsuccessful trial). At the end of a trial, the actuator was returned to position P1 and the door was closed.

Direct control of BMI by M1 units

We observed that over the course of a typical 1-2h practice session, animals showed improvements in task performance with a significant reduction in the time to successful
trial completion and decrease in the proportion of unsuccessful trials. We report results here from 23 sessions, where we saw significant reductions in both metrics.

Overall we observed that rats showed improvement in task-performance with a significant reduction in the time to a successful trial completion (Fig. 1c,d; 7.69 ± 0.42 sec to 4.09 ± 0.3 sec, mixed-effects model: t(44) = -7.13, P = 7.3 × 10^{-9}) and a decrease in the percentage of unsuccessful trials (Fig. 1d; 28.12 ± 3.65 % to 2.71 ± 0.97 %, mixed-effects model: t(44) = -7.01, P = 1.0 × 10^{-8}).

In a subset of sessions, we performed video recording of rats during the BMI training and quantified the proficiency of brain control by ruling out movement of the implanted limb. Consistent with previous reports of lack of muscle contractions during neuroprosthetic control, we did not observe limb movements to systematically predict feeding tube movements^{15}. Specifically, we analyzed whether limb movements measured using the video recording correlated with movements of the feeding tube. Across multiple sessions we observed significant reduction of this correlation (Supplementary Fig 3a,b; 0.014 ± 0.002 to 0.006 ± 0.001, mixed-effects model: t(26) = -3.52, P = 1 × 10^{-3}).

We also analyzed the proportion of TR_{d}'s that developed robust task-related modulation (see Methods). Figure 1e, depicts the increase in the activity of M1 direct units from early to late trials. Since the direct units were causally linked to the movement of the actuator, we found that a high proportion of these units developed task-related modulation (96% of 50 TR_{d}'s), as has been reported in the past^{11}. 
Indirect modulation of neurons in M1 and cerebellum

We also analyzed indirect units in the M1 and cerebellum and checked for their task-related modulation. We further sub-classified indirect units as either task-related (TR’s) or task-unrelated (TU’s) based on changes in modulation depth with learning. Consistent with previous reports of indirect units in the M1 likely contributing to neuroprosthetic control\textsuperscript{11,13,15,19,21}, we found strong indirect modulation in M1 (Fig. 1f). Additionally, we found robust indirect modulation of units in the cerebellum (Fig. 1h). Majority of units in M1 and cerebellum developed strong indirect task-related modulation with learning (Fig. 1j). We also looked at the change in modulation depth (\(MD_\Delta\)) and found that M1 \(TR_d\) undergoes greater \(MD_\Delta\) from early to late trials as compared to M1 and Cb \(TR_i\) units (M1 \(TR_d\): \(130.46 \pm 47.8\%\); M1 \(TR_i\): \(51.075 \pm 15.5\%\); cerebellum \(TR_i\): \(25.88 \pm 9.5\%\), Kruskal-Wallis H-test, \(F_{2,713} = 195.23, P = 4.0 \times 10^{-43}\), post hoc \(t\)-test showed significant difference among M1 \(TR_d\), \(TR_i\) and cerebellum \(TR_i\) \(MD_\Delta\) ’s; \(P < 0.05\)). Our next analyses focused on these task-related cells.
Figure 1. Direct and indirect modulation of M1 and cerebellar activity with neuroprosthetic learning. 

**a**, Direct neural control of a feeding tube (θ = angular position). Each trial started with the tube at P1. **b**, Trial started with an audio tone cue and opening of the door. A successful trial required movement of the tube to P2 within 15 seconds. **c**, Change in task completion time as a function of trial number. Line shows moving average of 5 trials. Dots show raw task completion times across all trials. **d**, Change in time to trial completion (left) and reduction in the percentage of unsuccessful trials (right) from early to late trials across all sessions. **e**, Peri-event histogram (PETH) from early and late trials from a single session are shown in left and right panels respectively, M1 TRd task-related direct units. **f**, M1 TRi units. **g**, Cerebellum TRi units. 

**h**, Distribution of units classified as TRd, TRi and TU in M1 (top) and cerebellum (bottom). 

***p<0.001.
Emergence of coordinated task-related activity in M1 and cerebellar LFPs

Interestingly, as the rats became proficient in M1-driven neuroprosthetic control, we observed that a coordinated low-frequency activity (approximately 3–6 Hz, Fig. 2a) emerged with learning in both M1 and cerebellum. The task-related LFP power between 3–6 Hz increased from early to late trials in both M1 (Fig. 2b,c; 0.44 ± 0.07 to 0.80 ± 0.075, mixed-effects model: \( t(44) = 4.9, P = 1.0 \times 10^{-5} \)) and the cerebellum (Fig. 2d,e; 0.18 ± 0.03 to 0.37 ± 0.04, mixed-effects model: \( t(44) = 2.5, P = 1.0 \times 10^{-2} \)). Task-related LFP coherence also increased in the 3–6 Hz frequency range from early to late trials (Fig. 2f, g; 0.16 ± 0.01 to 0.22 ± 0.013, mixed-effects model: \( t(44) = 4.6, P = 2.5 \times 10^{-5} \)). This emergence of cross-region low-frequency activity is consistent with other observations of emergence of low-frequency activity during motor skill learning across reciprocally connected neural networks\(^7\).
**Figure 2. Co-ordinated task-related oscillations emerge in M1 and cerebellar LFPs**

**a,** Raw and filtered LFP trace from a representative trials showing increase in 3–6 Hz oscillations after task-start during late trials in both M1 and cerebellum. **b,** Spectrogram from a representative M1 channel showing increase in 3–6 Hz power during late trials. **c,** Increase in 3–6 Hz power in M1 LFP from early to late trials across sessions. **d,** Same as **b** but from a representative cerebellum LFP channel. **e,** Same as **c** for 3–6 Hz cerebellum LFP power across sessions. **f,** Coherogram from a representative pair of M1–cerebellum LFP channel showing increase in 3–6 Hz coherence during late trials. **g,** Change in 3–6 Hz coherence from early to late trials across sessions. *p<0.05, ***p<0.001.
Increase in locking between spiking and LFP after neuroprosthetic learning

We next investigated the relationship between spiking activity and the low frequency LFP oscillations that we observed during robust task engagement. We performed spike-triggered averaging (STA) of the LFP in early and late learning time-locked to spikes occurring either in the same region (i.e., M1 spikes to M1 LFP and cerebellum spikes to cerebellum LFP) or in the other region (i.e., M1 spikes to cerebellum LFP and cerebellum spikes to M1 LFP). If spiking activity was independent of low-frequency LFP activity, then fluctuations would cancel and produce a flat average LFP, and hence, the STA can provide an intuitive estimate of how neural spiking is modulated by the low-frequency LFP oscillations. We observed a clear increase in the amplitude of mean LFP oscillations in both regions around action potentials of M1 $TR_d$, $TR_i$ and cerebellum $TR_i$ units (Fig. 3a).

This increase was not present for these classes of cells when we did the STA analysis during task-unrelated inter-trial interval (Fig. 3b). M1 $TR_d$ and $TR_i$ units, and cerebellum $TR_i$ units experienced 86.15 ± 11.50%, 90.97 ± 3.78% and 83.28 ± 5.95% increases in STA amplitude with M1 LFP during task periods, respectively. During inter-trial interval these units experience a decrease of −3.52 ± 2.68%, −0.25 ± 2.55% and −2.02 ± 2.39%, respectively (Fig. 3c; Kruskal-Wallis H-test, $F_{5,137} = 73.74$, $P = 3.0 \times 10^{-20}$, post hoc $t$-test showed significant difference for M1 $TR_d$, $TR_i$ and cerebellum $TR_i$ during task-relevant period and inter-trial period; $P < 0.001$). When STA was performed with cerebellum LFP, M1 $TR_d$ and $TR_i$ units, and cerebellum $TR_i$ units experienced 80.87 ± 11.28%, 78.52 ± 6.83% and 113.75 ± 22.10% increases in STA amplitude (during the task period), respectively. Similar to M1 LFP, these units experienced significantly less change of 2.55 ± 1.73%, −0.81 ± 2.85% and 1.44 ± 3.52%, respectively, when STA was performed with
cerebellum LFP during inter-trial interval (Fig. 3d; Kruskal-Wallis H-test, $F_{5,137} = 23.63$, $P = 5.6 \times 10^{-21}$, post hoc t-test showed significant difference for M1 $TR_d$, $TR_i$ and cerebellum $TR_i$ during task-relevant period and inter-trial periods; $P < 0.001$).

Figure 3. M1 and cerebellum spike-LFP locking increases with learning. a, The mean M1 LFP (top row) or cerebellum LFP (bottom row) time locked to occurrences of spikes from M1 (left column) or cerebellum (right column) during task-period from a representative session. b, Same as a during inter-trial interval. c, Percentage change in STA amplitude for M1 LFP in each of the categories of units (mean ± s.e.m.). d, Same as c for STA of cerebellum LFP. ***$p<0.001$. 

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Increase in neural synchrony between M1 and cerebellum

We subsequently assessed whether there are changes in the correlated spiking among the recorded M1 and cerebellum neural ensembles with neuroprosthetic learning. To analyze this, we looked at how the magnitude of cross-correlation between pairs of M1 TRd’s and cerebellum TRi’s changed. An example of an increase in cross-correlation is shown in Fig. 4a. At the population level, we calculated the magnitude of the difference between the peak average early trials and late trials correlogram from a shuffled correlogram (ΔCCH, see "Methods"). We found a significant increase in correlated firing (calculated as an increase in above-mentioned difference) during late trials (Fig. 4b; 4.52 ± 0.46 to 6.30 ± 0.60, mixed-effects model: t(44) = 5.15, P = 5.8 × 10⁻⁶). Thus, our findings show that ‘direct’ units in M1 (M1 TRd’s) and ‘indirect’ cerebellar TRi’s fire more coincidently with neuroprosthetic learning.

We also performed cross-correlation between M1 TRd’s and M1 TRi’s. Consistent with previous reports¹¹,¹⁵, we found a significant increase in the magnitude of cross-correlation (Fig. 4c; 2.86 ± 0.41 to 4.18 ± 0.46, mixed-effects model: t(44) = 2.64, P = 0.01). We also performed cross-correlation between M1 TRi’s and cerebellum TRi’s (see Fig. 4d). Surprisingly, we found no significant change in the magnitude of cross-correlation in this pair with learning (Fig. 4e; 3.56 ± 0.69 to 3.11 ± 0.54, mixed-effects model: t(44) = −1.92, P = 0.06).
Figure 4. M1 TR\textsubscript{d} and cerebellar TR\textsubscript{i} neural synchrony increases with learning. 

*a*, Representative cross-correlogram of a pair of M1 TR\textsubscript{d} and cerebellum TR\textsubscript{i} unit in early (left) and late (right) trials. 

*b*, Change in magnitude of cross-correlogram from early to late trials across all sessions for all M1 TR\textsubscript{d} and cerebellum TR\textsubscript{i} units (mean ± s.e.m.). 

*c*, Same as *b* for M1 TR\textsubscript{d} and M1 TR\textsubscript{i} units pairs. 

*d*, Representative cross-correlogram of a pair of M1 TR\textsubscript{i} and cerebellum TR\textsubscript{i} unit in early (left) and late (right) trials. 

*e*, Same as *b* for M1 TR\textsubscript{i} and cerebellum TR\textsubscript{i} unit pairs. 

*p<0.05 ***p<0.001

Fine timescale coordination of M1 and cerebellum activity with task learning

While our analyses so far show a coordinated activity in M1 and cerebellum with task learning; it doesn’t necessarily indicate that the neural activity patterns in the two structures are coordinated across trials. Recent studies have explored fine-timescale coordination at the level of spiking\textsuperscript{36,37}. Such methods use statistical methods to measure ‘communication subspaces’ based on ensemble patterns. Here, we used canonical correlation analysis (CCA) to assess fine-timescale coordination between M1 and cerebellum. CCA has been used in various neuroscience studies to extract correlated...
population activity between two areas$^{36–40}$. Specifically, CCA finds a linear combination
of units in M1 and cerebellum that project to M1 and cerebellum subspaces, respectively,
and where activity in these subspaces is maximally correlated (Fig. 5a). We used
concatenated single-trial spiking activity binned at 100ms as in two recent papers$^{37,40}$.
The top component produced by CCA is the axis of the M1 and cerebellar subspaces that
has the maximum correlation between the two areas. We found that this maximum
correlation increased with neuroprosthetic learning only for M1 TR$\text{d}$ and cerebellum TR$\text{i}$
units (Fig. 5b shows CCA changes in an example session from a single animal, and Fig.
5c shows all sessions from all animals; early canonical correlation: 0.24 ± 0.032, late
canonical correlation 0.54 ± 0.035, mixed-effects model: $t(44) = 7.55, \ P = 1.7 \times 10^{-9}$).
Moreover, we found that the subspace activity in the two structures became more
precisely temporally correlated with learning (Fig. 5d). For M1 TR$\text{i}$ 's and cerebellar TR$\text{i}$
's, maximal correlation produced by CCA didn’t show a significant increase
(Supplementary Fig. 4; 0.37 ± 0.042 to 0.39 ± 0.045, mixed-effects model: $t(44) = 0.52,
\ P = 0.604$).
Figure 5. Increase in neural subspace correlation between M1 and cerebellum. 

**a,** Description of canonical correlation analysis (CCA). CCA finds a linear combination of binned spike counts from M1 units ($x_1, x_2, \ldots, x_n$) and cerebellum units ($y_1, y_2, \ldots, y_n$) that maximizes the correlation between M1 and cerebellum.

**b,** M1 $TR_d$ and cerebellum $TR_i$ subspace activity (from the first canonical component) around task-start ($-2$ to $2$ s) for an example session. Each dot represents one time bin of early or late trials from the session. Canonical correlation score is given by $r$. 

**c,** Change in the canonical correlation score from early to late trials across all sessions. ***$p<0.001$. 

**d,** Relationship between CCA correlation score and average time to a successful trial across all sessions.
Cerebellar indirect modulation leads M1 response after task onset

With recent reports of M1 activity being input driven and secondary motor cortex’s (M2) modulatory influence over M1 during M1-driven neuroprosthetic task\textsuperscript{37,41}, we wanted to check how did the cerebellar TR\textsubscript{i} activity ‘integrate’ with M1 TR\textsubscript{d} activity. Interestingly, both early and late in learning, the temporal profiles of modulation (i.e., Fig. 6a) for M1 TR\textsubscript{d} and cerebellar TR\textsubscript{i} were different, suggesting that these two distinct groups of task-related units became functionally and temporally coupled with learning and successful task performance. Additionally, we also observed that cerebellum TR\textsubscript{i} activity tended to peak before M1 TR\textsubscript{d} during early trials (Fig. 6b; time to peak for M1 activity: 252.27 ± 15.53 ms and time to peak for cerebellum activity: 228.08 ± 14.18 ms, mixed-effects model: \textit{t}(332) = −1.20, \textit{P} = 0.23) and late trials (Fig. 6c; time to peak for M1 activity: 249.92 ± 5.99 ms and time to peak for cerebellum activity: 231.51 ± 5.86 ms, mixed-effects model: \textit{t}(332) = −2.19, \textit{P} = 0.02).
Figure 6. Cerebellum TR activity peaks before M1. a, PETH showing the difference of time-to-peak for a M1 TRd and cerebellum TRi units during early trials. b, Same as a for late trials. c, Difference between time to peak across all M1 TRd and cerebellum TRd units during early trials. d, Same as c for late trials. n.s. indicates p>0.05 *p<0.05.

Cerebellar optogenetic inhibition impairs neuroprosthetic performance

Next, we wanted to see the effects of optogenetic inhibition of cerebellum and the effects on neuroprosthetic skill learning. We used a red-light shifted halorhodopsin- JAWS for inhibiting neural activity in the cerebellum (see Methods). First, we found that JAWS was robustly expressed in cerebellar cortical neurons (Fig. 7a). When we looked at the activity of cerebellar neurons under optical illumination acutely, we found that JAWS activation
led to strong inhibition of cerebellar neurons (Supplementary Fig. 5a,b). Optogenetic inhibition significantly reduced firing across cerebellar neurons (Supplementary Fig. 5c; Stimpre: 27.30 ± 2.88 Hz, StimON: 4.99 ± 0.67 Hz and Stimpost: 20.16 ± 2.09 Hz; Stimpre vs StimON mixed-effects model: t(88) = −7.69, P = 1.9 × 10−11; Stimpost vs StimON mixed-effects model: t(88) = 7.05, P = 3.8 × 10−10), with a reduction in 84.44% of recorded cells during StimON; (n = 45).

Next, we performed optogenetic inhibition of the cerebellum in chronically implanted rats during the BMI task practice (see Methods). When we inhibited the cerebellum in rats that had already gained proficiency in neuroprosthetic task performance (i.e., during late trials), we found that time to successful completion of neuroprosthetic task increased (Fig. 7b; 5.95 ± 0.68 s without cerebellum inhibition and 8.85 ± 0.69 s with cerebellum inhibition, mixed-effects model: t(22) = 3.24, P = 3.7 × 10−3). Furthermore, we also found that cerebellar inhibition decreased the MDΔ of M1 TRd units during trials when cerebellum was optogenetically inhibited (Fig. 7c,d; 190.11 ± 49.86% without cerebellum inhibition and 112.11 ± 17.42% with cerebellum inhibition, mixed-effects model: t(34) = −1.71, P = 1 × 10−2). Interestingly, this effect of cerebellar inhibition was not as strong on M1 TRi units as it was on M1 TRd units (Supplementary Fig. 6; MDΔ for M1 TRi units: 48.67 ± 6.90% without cerebellum inhibition and 47.13 ± 4.77% with cerebellum inhibition, mixed-effects model: t(391) = −0.15, P = 0.87). Cerebellar inhibition also impacted M1 3−6 Hz LFP power. During late trials, 3−6 Hz M1 power was reduced under cerebellar inhibition (Fig. 7e,f; 1.29 ± 0.14 z-scored M1 power without cerebellum inhibition and
0.73 ± 0.11 M1 power with cerebellum inhibition, mixed-effects model: \( t(22) = -3.30, P = 3.2 \times 10^{-3} \).

While these experiments spoke about loss of performance on the neuroprosthetic task when cerebellum was optogenetically inhibited, we also observed that the effects of cerebellar inhibition on neuroprosthetic learning was not related to the order in which it was performed, i.e., effects of inhibition of cerebellum in first BMI block versus the second BMI block within a day with the same M1 \( TR_d \) units (see Methods for details on the order of two BMI blocks within a day), Supplementary Fig. 7b; time to task completion with cerebellum inhibition in first BMI block: 9.33 ± 0.86s versus time to task completion without cerebellum inhibition in second BMI block: 6.34 ± 1.02s, mixed-effects model: \( t(12) = -2.58, P = 2 \times 10^{-2} \). Supplementary Fig. 7c; Time to task completion without cerebellum inhibition in first BMI block: 5.54 ± 0.9s versus time to task completion with cerebellum inhibition in second BMI block: 7.98 ± 1.49s, mixed-effects model: \( t(8) = 1.74, P = 1 \times 10^{-2} \). Hence, our results show that cerebellum was actively involved in supporting rapid M1-driven neuroprosthetic control, whether the animals were new to the BMI control scheme or when they had become proficient with a given control scheme.
Figure 7. BMI performance gets impaired with cerebellar inhibition

a, Fluorescence image of a coronal brain section showing neurons expressing JAWS (green) in the cerebellum cortex.

b, Cerebellar inhibition increases time to task completion. ***p<0.001.

c, PETH of an example M1 TRd unit, during late trials, with (Laser ON) and without (Laser OFF) cerebellar inhibition. 

d, Change in modulation depth (MDd) of M1 TRd units from early to late trials, with and without cerebellar inhibition. *p<0.05.

f, The 3-6 Hz power emerges during late trials on the same day session where cerebellar inhibition was not done.
Discussion

In this study, we found an emergent 3-6Hz activity in the M1 and cerebellum LFPs that also modulated task-related direct and indirect spiking in these regions. Additionally, we found that neuroprosthetic learning led to increased synchronous spiking between M1 TRd and cerebellar TRi units, and this pair also had a stronger spiking coordination across trials as indicated by heightened canonical correlation with learning. Furthermore, optogenetic inhibition of the cerebellum led to performance impairments (with increased time to task completion) and weakening of M1 TRd activity. At population spiking level, we also found that cerebellar activity was leading M1 direct activity. These findings suggest that cerebellum plays an important hierarchical role by providing influence on M1 neural patterns that are related to behavioral output. Furthermore, we found that cerebellar indirect activity became more synchronous with M1 direct activity and canonical correlation also increased between M1 TRd and cerebellum TRi's, but this was not observed for M1 TRi and cerebellum TRi units suggesting that cerebellum had a more privileged relationship with effector-specific neurons of M1. Although we found that M1 TRi's correlated activity increased with M1 TRd units, indicating that same-area interactions also participate in achieving neuroprosthetic skill learning, cerebellum TRi's preferential coordinated activity with only M1 direct units suggests that cerebellum has a strong selective online modulatory influence on M1, as well as instantiating behaviorally relevant patterns. This role of cerebellum that we found in neuroprosthetic skill learning is consistent with cerebellum's role in fine-tuning movement, as well as co-emergent activity patterns that have been reported with learning new skills\textsuperscript{2,42}. Our studies here have helped elucidate cerebellar contributions to M1-driven neuroprosthetic learning and
can help improve BMI functionality in the future. For example, BMI paradigms could incorporate cerebellar indirect signals for improving BMI controllers.

**Emergent mesoscopic dynamics across M1-cerebellum**

One of our first findings was an emergence of 3-6 Hz coherence in M1-cerebellum LFPs associated with learning, and neurons in both these regions also showed enhanced phase-locking to this oscillation during task-relevant periods as revealed through STA. Similar observations have been observed in a neuroprosthetic study that looked at task-related cells in cortico-striatal networks\(^{20}\). Such coherence can serve to enhance communication during task period between task-relevant cell populations across the larger motor networks that should integrate signals for optimal behavioral output. Such coherent activity may allow for flexible use of task-relevant cells in either region. The synchrony that we observed in 3-6 Hz band is consistent with other work that has showed low-frequency coherence between M1 and other motor regions during learning\(^{6,7}\).

**Using BMIs to study cross-region coordination in motor control.**

BMI offers investigations of connected regions by selecting target neural patterns (that dictate output) in one region and concurrent examination of another region as task performance improves. Implementing this strategy, we aimed to disentangle cerebello-cortical communication as M1 direct control was learned. Importantly, both M1 and cerebellum have direct connections to the spinal cord\(^{8-10}\), and are implicated in movement control\(^{3,4,6}\). In our BMI paradigm, we randomly selected target neural pattern in M1...
(enforced by the decoder), which is unlikely to be correlated with processes in other brain
regions. This permitted us to examine how cross-area communication during BMI control
facilitates control. M1 and cerebellum are reciprocally connected\(^1,2\), and while some
extent of coupling of task-related activity in M1 and cerebellum is not surprising, it is
unknown how the indirect cerebellum activity interacts with behavioral output specific
direct activity versus indirect or non-task specific activity of M1 neurons.

One of the striking findings of our work is that canonical correlation increased between
M1 \(TR_d\) and cerebellum \(TR_i\) units. Similarly, cross-correlograms between these unit pairs
also showed more coincident firing. However, this was not the case for M1 \(TR_i\)'s and
cerebellum \(TR_i\)'s. Cerebellum optogenetic inhibition also weakened modulation of M1 \(TR_d\)
's which suggests that the cerebellum communicated more strongly with task-specific
pools of M1 direct activity. These ideas are consistent with theoretical studies that
suggest that cerebellar cortex helps in task-relevant dimensionality expansion that aids
in learning\(^43\).

**Roles of multiplexed cross-area interactions**

Motor control involves signals at longer time scales appropriately integrating with shorter
time scales across several spatially segregated regions to deliver movement precision\(^44\).
Little is known about how these signals at varying spatiotemporal scales are interacting
during motor control. Simultaneous recordings are best suited to understand these
interactions\(^6,36,36,45–47\). Further, a majority of studies that have used simultaneous
recordings in motor regions use extensively trained animals performing natural motor
tasks. Understanding M1-cerebellum interactions in such context raises important concerns: (i) since both M1 and cerebellum are directly controlling movement, it is difficult to ascertain modulatory influence of one area over the other; and (ii) overtrained animals may have transitioned to an ‘automatic’ state, and it may no longer be suitable to investigate emergent dynamics in interacting structures. These confounds limit the inferences about M1-cerebellum interactions in experiments with extensively trained animals.

Here we have shown that in an M1-driven BMI task, cerebellum activity leads M1 direct activity and hence, may play an instructional role in the beginning. This idea is consistent with the notion of cerebellar role in skill acquisition. We find that cerebellar indirect activity leads later in learning as well, indicative of an ongoing modulatory influence over M1 to sustain proficiency in the task. This is consistent with the notions of cerebellar contributing to fine-tuning effector movements. We also find that M1 indirect activity is correlated to M1 direct activity, but not to cerebellar indirect activity. This leads us to conclude that M1 output-specific neurons contain multiplexed signals coordinated with both local and distant-area activity and that cerebellar activity might be somatotopically organized; thus, the cerebellar activity is retained in the cortico-cerebellar loop. Our cross-correlation between M1 $TR_d$ and cerebellum $TR_i$ units also showed a broader timescale influence of cerebellum on M1 activity (this is different from the shorter timescale influences seen between M1 $TR_d$ and M1 $TR_i$ units). Such broader influence of cerebellar activity may be related to coordination in the larger motor network. Larger
network activity may represent attention regulation\textsuperscript{54}, motivation\textsuperscript{55} or coordination of the motor task with sensory feedback\textsuperscript{56}.

479 **Neural dynamics over the course of BMI learning**

Natural motor learning is known to involve an early phase marked exploration and high variability with a transition to late stage when the skill is consolidated\textsuperscript{57–60}. Our paradigm here is focused on early exploratory BMI learning by using mostly single-sessions. BMI studies that allow for sleep consolidation\textsuperscript{11,12,18} or use multiple days of learning\textsuperscript{15,61} find that M1 TR\textsubscript{i} units weaken their modulation through the course of extensive training. It is possible that cerebellar TR\textsubscript{i}’s may exhibit similar weakening over time. However, local versus cross-area interactions may differ in the long-term. One recent study had focused on cross-area activity through multiple days of training, and they found that indirect task-related modulation persists in several cortical areas\textsuperscript{23}. Work that has looked at extensively trained mice on motor task has shown sustained activity in the cerebellum\textsuperscript{42}. There is further evidence from studies of natural learning that emergent activity in cortico-cerebellar networks becomes stronger as task proficiency increases\textsuperscript{7}.

To summarize, our studies leveraged a multiarea BMI paradigm to probe M1-cerebellar cross-area interactions. We demonstrated that oscillatory dynamics emerged as seen through LFPs across these regions that also modulated task-related spiking in both areas. Finer time scale analyses of spiking revealed that cerebellar indirect activity selectively influenced our imposed artificial target activity pattern within M1. This paradigm allowed us to manufacture an output-specific pattern, with a relatively small neural footprint to
499 examine internal motor networks dynamics, removing the constraints of movement
500 performance. Thus, multiarea BMIs, through such impositions, allows for a more natural
501 inside-out investigation of cross-area interactions.
Methods

Animal preparation

Adult male Long-Evans rats were used in this study (n = 13, 250–400 g, ~8 weeks old, Charles River Laboratories). All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center, Los Angeles. This ensured that the animals that were used in this research were acquired, cared for, housed, used, and disposed of in compliance with the applicable federal, state, and local laws and regulations, institutional policies and with international conventions to which the United States is a party. Animals were housed on a 14 h light and 10 h dark cycle (Photoperiod is from 6 am to 8 pm) in a climate-controlled vivarium. Out of 13 rats, 8 were used in BMI with simultaneous M1 and cerebellum recordings, 3 rats were used for BMI with cerebellar optogenetic inhibition and the remaining (n = 2) were used in acute cerebellar recording under optogenetic inhibition. Neural probes were implanted during a recovery surgery performed under isofluorane (1–3%) anesthesia. The analgesic regimen included the administration of 0.1 mg per kg body weight buprenorphine, and 5 mg per kg body weight carprofen. Post-operatively, rats were also administered 2 mg per kg body weight dexamethasone and 33 mg per kg body weight sulfatrim for 5 days. Ground and reference screws were implanted posterior to lambda contralateral to the recorded cerebellum, contralateral to the neural recordings. For M1 recordings, 32-channel arrays (33-μm polyamide-coated tungsten microwire arrays) were lowered to a depth of ~1,200–1,500 μm in either the left or right M1 depending on handedness. These were implanted centered at 0.5 mm anterior and 3 mm lateral to the bregma (Ramanathan et al., 2015; Lemke et al., 2019). For cerebellar
recordings we used 32-64 channel tetrodes (Neuronexus, MI) or shuttle-mounted polytrodes (Cambridge Neurophysiology, UK). The probes were lowered into the cerebellar cortex through a craniotomy centered at 12.5 mm posterior and 2.5-3 mm lateral to bregma. Shuttle mounted probes were moved across days and recorded from depths of 1.5-4 mm. Our target regions were Simplex/ Crus I and Crus II areas of the cerebellum (Atkins and Apps, 1997; Baker et al., 2001; Heck et al., 2007). Activity in these areas has shown modulation during upper limb motor behaviors in response to corticofugal fiber and forelimb stimulation. We did not perform subject-specific implantation based on motor mapping. However, a subset of rats also performed reaching tasks and had robust activation during reaching.

Viral injections

We used a red-shifted halorhodopsin, Jaws (AAV8-hSyn-Jaws-KGC-GFP-ER2, UNC Viral Core) for neural silencing in 5 rats for optogenetic experiments. Viral injections were done at least 3 weeks before chronic implant surgeries. Rats were anesthetized, as stated before and body temperature was maintained at 37 °C with a heating pad. Burr hole craniotomies were performed over injection sites, and the virus was injected using a Hamilton Syringe with 34G needle. 500-nl injections (100 nl per min) were made at two sites in the cerebellum (11.5 mm posterior, 2.5 mm lateral to bregma and 11.5 mm posterior and 3.5 mm lateral to bregma; depth of 1-3 mm). After the injections, the skin was sutured, and the animals were allowed to recover with same regimen as stated above. Viral expression was confirmed with fluorescence imaging.
Electrophysiology

Units and LFP activity were recorded using a 128-channel TDT-RZ2 system (Tucker-Davis Technologies). Spike data were sampled at 24,414 Hz and LFP data at 1,017.3 Hz. ZIF (zero insertion force) clip-based digital head stages from TDT were used that interface the ZIF connector and the Intan RHD2000 chip that uses 192x gain. Only clearly identifiable units with good waveforms and high signal-to-noise were used. The remaining neural data was recorded for offline analysis. Behavior related timestamps (i.e. trial onset, trial completion) were sent to the RZ2 analog input channel using an Arduino digital board and synchronized to neural data.

We have used the term ‘unit’ to refer to the sorted spike recordings from both the MEA and silicon probe recordings. For both, we initially used an online sorting tool (Synapse, TDT) for neuroprosthetic control. We used waveform shape and the presence of an absolute/relative refractory period in the inter-spike interval (ISI) to judge quality of isolation. Specifically, a voltage-based threshold was set based on visual inspection for each channel that allowed for best separation between putative spikes and noise; typically, this threshold was at least 4 standard deviations (SD) away from the mean. Events were time-stamped and waveforms for each event were peak aligned. K-means clustering was then performed across the entire data matrix of waveforms. Automated sorting was performed by: (1) first over-clustering waveforms using a K-means algorithm (i.e., split into many mini-clusters), (2) followed by a calculation of interface energy (a nonlinear similarity metric that allows for an automated decision of whether mini-clusters
are actually part of the same cluster), and (3) followed by aggregation of similar clusters.

We conducted offline spike sorting in Plexon or Spyking Circus\textsuperscript{63}.

**Behavior**

After recovery, animals were typically acclimated for 1-2 day to a custom plexiglass behavioral box (Fig. 1a) prior to the start of experimental sessions. After acclimatization, rats were water restricted for BMI training. We monitored body weights daily to ensure that the weight did not drop below 95\% of the initial weight.

Behavioral sessions were typically conducted for 1-2 hours. Recorded neural data was entered in real-time to custom routines in Matlab (R2018b; Mathworks, Natick, MA). These then served as control signals for the angular velocity of the feeding tube. The rats performed \(~120\) trials on an average in a session (e.g. Fig. 1c). In a subset of sessions (n = 14), we also video-monitored the rat during the BMI training using a 30-fps camera (TDT, USA).

**Neural control of the feeding tube**

During the BMI training sessions, we typically selected one or two M1 channels’ units to be selected as ‘direct’ (\textit{TR}_d) units, and their neural activity was used to control the angular velocity of the feeding tube. If one channel was choosen for neuroprosthetic control, its neurons were associated with positive weight and if two channels were chosen, one channels neurons were associated with a positive weight and another’s with a negative weight. These units maintained their stability throughout the recording as evidenced by
stability of waveform shape and interspike-interval histograms (Supplementary Fig. 1).

We binned the spiking activity into 100ms bins. We then established a mean firing rate for each neuron over a 3–5 minute baseline period. The mean firing rate was then subtracted from its current firing rate at all time points. The specific transform that we used was:

$$\theta_v = C \times [G_1 \times r_1(i) + G_2 \times r_2(i)]$$

where $\theta_v$ was the angular velocity of the feeding tube, $r_1(i)$ and $r_2(i)$ were firing rates of the direct units. $G_1$ and $G_2$ were randomized coefficients that ranged from +1 to −1 and were held constant after initialization. $C$ was a fixed constant that scaled the firing rates to angular velocity. The animals were then allowed to control the feeding tube via modulation of neural activity. The tube started at the same position at the start of each trial ($P_1$ in Fig. 1a). The calculated angular velocity was added to the previous angular position at each time step (100 ms). During each trial, the angular position could range from −45 to +180 degrees. If the tube stayed in the ‘target zone’ ($P_2$ in Fig. 1a; spanned 10° area) for a period of 400 ms, then a water reward was delivered. In the beginning of a session, most rats were unsuccessful at bringing the feeding tube to position $P_2$. Most rats steadily improved control and reduced the time to completion of the task during a session. Multiple learning sessions were obtained from each animal. Consistent with past studies, we also found that incorporation of new units into the control scheme required new learning\textsuperscript{11,64–66}.
Optogenetics

Optogenetic experiments was carried out in JAWS injected rats using a high-power laser (50 W/mm²: Laserglow Technologies, USA) emitting a 625 nm beam. A subset of rats (n = 3) was implanted with a cannula over Crus I/Crus II region of the cerebellar cortex and a 32-channel microwire array (TDT Florida) was implanted in M1. During the behavior experiments, laser was turned on at every 10 ms at 50% duty cycle to inhibit cerebellar activity after the trial onset for the total duration of the trial (15s). We performed two sessions each day with ~100 trials in each session. Each day, we alternated between turning the laser on in the first session or the second session (Supplementary Fig. 6a).

Another subset of rats (n = 2) was implanted with fiber-optic cannula mounted on a silicon probe (Cambridge Neurotech, UK) in the cerebellum, and the recordings were performed under isofluorane anesthesia. In every trial, we recorded 5s of baseline followed by 5s for which the laser was on and another 5 s thereafter with laser off. We performed 30 such repetitions.

Histology

After the experiment, rats were deeply anaesthetized with isoflurane (4-5%), then exsanguinated and perfused with 4 % paraformaldehyde (PFA). The brains were extracted and stored in 4% PFA for up to 72 hours. The brains were then transferred to a solution of 30% sucrose and stored for sectioning. We performed sagittal or coronal sections of the brain using a cryostat (Leica, Germany) and stored them in phosphate-buffered saline for imaging. Images were mounted on slices and imaged using a
microscope (Keyence, Japan). The location and depth of the silicon probe in the brain were traced by Dil depositing on the electrodes prior to their implantation and by looking afterwards at the fluorescent dye present in the histological slices (Supplementary Fig. 2).

Data Analysis

Sessions and changes in performance

Analysis was performed in Matlab (R2020b) with custom-written routines. A total of 23 training sessions recorded from 8 rats were used for our initial analysis. In addition, we analyzed 12 separate sessions across 3 rats where optogenetic inhibition of the cerebellum was performed. For Fig. 1b,c we compared changes in task performance across sessions. Specifically, we compared the performance change by calculating the mean and standard error of the mean (s.e.m) of the time to completion during the first and last 30% of trials (referred as early and late trials respectively). Furthermore, we also compared the best performance in early and late trials by calculating the percentage of unsuccessful trials.

Task-related activity

The distinction between TRd, TRi and TU units was based on whether significant modulation of baseline firing activity after trial onset (i.e., peak of modulation at the time > 2.5 SD above the baseline period).
Artifact rejection was first performed on LFP signals to remove broken channels and noisy trials. LFPs were then z-scored, median referenced and evoked-activity was subtracted separately for M1 and cerebellum. LFP power was calculated on a trial-by-trial basis and then averaged across channels and animals, with wavelet decomposition using the EEGLAB function `newtime`\(^6\). M1-Cb LFP coherence was calculated for each pair of channels using the EEGLAB function `newcross`\(^6\). All the comparisons were done between early and late trials. For this analysis, across all the early trials, only trials where time to task completion was over 10 seconds were included. Across all the late trials, only trials where time to task completion was under 5 seconds were included.

**Spike-triggered averaging (STA)**

We calculated the STA to measure how spikes locked to the 3-6 Hz LFP oscillations, both in the M1 and the cerebellum. We used filtered (3–6Hz) median LFP from each region for this analysis. For filtering, we used the EEGLAB function `eegfilt`. We used the first 4 seconds after the start of the trial to calculate these STAs. For every unit, we concatenated the spikes from early trials in a spike vector and from late trials in another vector. Before STA calculation, we equaled the length these vectors. Then, we extracted 2 sec of LFP around every spike-time in those vectors and average it to get early and late STAs for a given unit. To calculate the change in modulation for every unit, we looked at the difference between the minimum and maximum peak in a 300 ms window around a spike in the averaged STA of early and late trials and then calculated this change from early to late trials in percentage. We also calculated STA during inter-trial interval. Here,
we used from 2 to 6 seconds after the end of the trial and applied the same steps to compute the STA as described above.

**Spike cross-correlation**

We computed the cross-correlation histogram (CCH) for M1 TR\textsubscript{d} – cerebellar TR\textsubscript{i}, M1 TR\textsubscript{i} – cerebellar TR\textsubscript{i} and M1 TR\textsubscript{d} - M1 TR\textsubscript{i} unit pairs. For every trial, the spike counts were equalized between the pairs before computing the CCH using 10 ms bins. Furthermore, we constructed pseudo-random spike train CCHs with simulated spike counts of equal length for every pairing. Such simulations were run 300 times (Monte Carlo Simulations)\textsuperscript{11,68}. To quantify pairwise coincident firing from early to late trials, we took the mean of the simulated CCH magnitude within ± 400 ms around the center and subtracted it from the mean of real CCH magnitude in the same window for early and late trials, respectively (ΔCCH).

**Canonical correlation analysis**

CCA identifies maximally correlated linear combinations between two groups of variables. Unit spiking data in M1 and cerebellum from -2 s to + 2 s around task-start for each trial were binned at 100ms and concatenated. CCA models were then fit using the MATLAB function `canoncorr`. Only the top canonical component was used in this paper.

**Video tracking & trajectory analysis**

We performed automated tracking of the tip of the feeding tube and the implanted forepaw of the rats using DeepLabCut\textsuperscript{69}. We did cross-correlation between the trajectories of the
feeding tube and the forepaw using \textit{xcorr} function of MATLAB and looked at the average cross-correlation magnitude $\pm$30ms around zero lag for every trial.

\textbf{Statistical analysis}

All statistical analyses were implemented within MATLAB. The linear mixed-effects model (implemented using MATLAB \textit{fitlme}) was used to compare the differences in behavior, trial to mean correlation, speed decoding and canonical correlation unless specified otherwise. This model accounts for the fact that units or sessions from the same animal are more correlated than those from different animals and is more stringent than computing statistical significance over all units and sessions\textsuperscript{6,40,70}. We fitted random intercepts for each rat and reported the $p$-values for the regression coefficients associated with sessions, channels, or units. We also performed Kruskal-Wallis H-test with multiple comparisons in \textbf{Fig. 3c} and \textbf{d}.
Supplementary Figure 1. Single Unit Waveforms and Inter-Spike Interval (ISI).  

**a**, Left panel shows waveforms of a representative M1 \( TR_d \) unit. Right panel shows ISI histograms for the same unit.  

**b**, Left panel shows waveforms of a representative task-related M1 \( TR_i \) unit. Right panel shows ISI histograms for the corresponding units in the left panel.
Supplementary Figure 2. Cerebellum electrode localization. The location of the electrode in lobule 7 of the cerebellum is indicated by a white arrow in the image of a brain section 4.75 mm lateral to the midline.
Supplementary Figure 3. Movement correlation reduces between forepaw and feeding tube. (a) Decrease in movement correlation in an example session. (b) Correlation reduces from early to late trials. **p<0.01.
Supplementary Figure 4. Canonical correlation analysis for M1 and cerebellum units. Change in CCA correlation shown between M1 and cerebellum task-related indirect (TRi) units. (n.s.; p>0.5).
Supplementary Figure 5. Inhibition of cerebellar neural activity with optogenetic illumination. 

**a**, A raw trace from an example channel showing reduction in activity when the laser was turned on. **b**, PSTH of an example unit showing reduction in firing rate with laser on. **c**, Change in the firing rate of all the recorded units before, during and after cerebellar inhibition. ***p<0.001.
Supplementary Figure 6. Effect of cerebellar inhibition on ‘indirect’ M1 units
Change in modulation depth ($MD_{\Delta}$) of M1 $TR_i$ units from early to late trials, with and without cerebellar inhibition. n.s.$p$>0.05.
Supplementary Figure 7. Cerebellar inhibition impairs BMI performance during task acquisition and maintenance. a, Schematic of session structure. On a day, initial session were performed with cerebellar inhibition trials (Laser ON) followed by subsequent session without cerebellar inhibition (top). Next day, the order was inverted with initial sessions’ trials without cerebellar inhibition performed first followed by subsequent trials with cerebellar inhibition (bottom). b and c, An increase in the time to task completion was observed irrespective of the order of cerebellar stimulation.
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Author Contributions

A.A. and T.G. designed the study. A.A., A.W.F. and N.P.D. carried out the electrophysiology experiments. A.A., A.W.F., N.P.D. and T.G. performed the surgical procedures. A.A. carried out the analysis. A.W.F. and N.P.D. assisted with analysis. A.W.F. and N.P.D. carried out histology. A.A. and T.G. wrote the manuscript. A.W.F. edited the manuscript.
References


Figure 1

(a) Schematic of the experimental setup showing the neural and behavioral components involved in the task.

(b) Time to task completion (s) for Early (E) and Late (L) trials.

(c) Graph showing the percentage of unsuccessful trials for Early (E) and Late (L) trials.

(d) Bar graph representing the time to task completion (s) for Early (E) and Late (L) trials.

(e) Firing rate (z-scored) for Early (E) and Late (L) trials.

(f) Graph depicting the firing rate (z-scored) for Early (E) and Late (L) trials.

(g) Graph illustrating firing rate (z-scored) for Early (E) and Late (L) trials.

(h) Pie chart showing the distribution of successful (TRd, TRi, TU) and unsuccessful trials (TRd, TRi, TU) with respective percentages.

Legend:
- TRd
- TRi
- TU

Parameters:
- n = 389 units
- n = 203 units

Note: The data points and statistical significance (*** indicates p < 0.001) are represented in the figure.
**Figure 3**

(a) Time-Locked to M1 TR\_d Spikes

(b) Time-Locked to Cerebellum TR\_i Spikes

(c) **Inter-trial Interval**

(d) **Inter-trial Interval**

- Mean M1 LFP
- Mean Cerebellum LFP

### STAamp Change (%)

- **M1 TR\_d**
- **M1 TR\_i**
- **Cerebellum TR\_i**

- **Early**
- **Late**

- 0.5 s
- 2 s.d.
Figure 4

a) Spike count over time for Early (E) and Late (L) conditions for M1 TRd and M1 TRi
d) Spike count over time for Early (E) and Late (L) conditions for Cerebellum TRi and TRd

b) ΔCCH for M1 TRd - Cerebellum TRi
   - Early (E): ***
   - Late (L): *

c) ΔCCH for M1 TRd - M1 TRi
   - Early (E): ****
   - Late (L): n.s.

d) ΔCCH for Cerebellum TRi - M1 TRi
   - Early (E): ****
   - Late (L): n.s.
Figure 5

(a) Canonical Correlation Analysis

Canonical variables $x_1, x_2, \ldots, x_n$ and $y_1, y_2, \ldots, y_n$ are maximally correlated with M1 and Cerebellar subspaces, respectively.

(b) Scatter plots showing CCA correlation for Early Trials ($r = 0.29$) and Late Trials ($r = 0.63$).

(c) CCA correlation for Early (E) and Late (L) trials.

(d) Scatter plot showing the relationship between CCA correlation and time to task completion ($T_{M1} - T_{Cerebellar}$), with $R^2 = 0.27$ and $P < 0.01$.
Figure 6

(a) Firing rate (z-scored) for M1 and Cerebellum over time after task-start. (b) Firing rate (z-scored) for M1 and Cerebellum showing distinct peaks during the early and late phases of the task.

(c) Bar graph showing the time after task-start for M1 and Cerebellum during the early phase with n.s. (not statistically significant). (d) Bar graph showing the time after task-start for M1 and Cerebellum during the late phase with a statistically significant difference indicated by an asterisk (*)

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Figure 7

- a: Image showing a brain slice with laser OFF and laser ON conditions.
- b: Graph showing time to task completion (s) for Laser OFF and Laser ON, with a significant difference indicated by an asterisk.
- c: Graph showing firing rate (Hz) over time (s) with task start indicated, showing an increase with Laser ON.
- d: Graph showing change in MΔ (%) for Laser OFF and Laser ON conditions, with a significant change indicated by an asterisk.
- e: Heatmap showing frequency (Hz) over power for Laser ON and Laser OFF conditions.
- f: Graph showing power nila for Laser OFF and Laser ON conditions, with a significant change indicated by three asterisks.
**Figure S1**

Counts vs. Time (ms) for two different traces. (a) Trace 1: 0.1 ms, 20 μV. (b) Trace 2: 900 Time (ms), Counts.

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Figure S3
Figure S4
Figure S6

The change in MDΔ (%): M1 TRi.

Change in MDΔ (%)

Laser OFF
Laser ON

n.s.
Figure S7

Task start | Time out (15 s) | Door close
---|---|---
Laser ON | | |

Task start | Time out (15 s) | Door close
---|---|---
Laser ON | | |

Task start | Time out (15 s) | Door close
---|---|---
Laser ON | | |

Laser ON

Time to task completion (s)

BMI1

BMI2

Time to task completion (s)

b

c

Time to task completion (s)

Laser ON

Laser OFF

*