Fastigial perturbation destroys the relationship between ALM activity trajectories and specific future movements

We examined the relationship of ALM activity trajectories to upcoming movements and the effect of fastigial photo-activation. ALM activity trajectories converge to discrete endpoints in activity space at the end of the delay epoch that correspond to specific future movements [1,2]. Future movement direction can be predicted from the distance of activity trajectories to these endpoints along the coding direction (***cd***). Previously, we found that bilateral photo-inhibition of ALM during early delay randomized future movement directions and resulted in chance-level performance (Extended Data Fig 10a-b). But in a trial-by-trial analysis movement direction could still be predicted based on the distance of the perturbed activity trajectories to the endpoints on the ***cd*** (Extended Data Fig 10c) [1,2]. Thus discrete endpoints in activity space represent specific movements and the endpoints are maintained even after a near complete silencing of ALM. We examined the relationship between ALM activity trajectories and future movements after fastigial photo-activation (Extended Data Fig 10b). We estimated the endpoints for “lick left” and “lick right” on the ***cd*** using activity trajectories from unperturbed trials. After fastigial photo-activation, the distance of the perturbed trajectories to these endpoints no longer predicted future movements (Extended Data Fig 10d). These analyses show that fastigial perturbation destroyed the relationship of ALM activity trajectories to specific future movements.

It is possible that fastigial perturbation activated downstream motor circuits that could maintain the motor plan and generate movements independent of ALM. We examined the necessity of ALM activity in driving directional licking after a fastigial perturbation. In VGAT-ChR2-EYFP mice expressing ChR2 in both Purkinje neurons and cortical GABAergic neurons, we independently manipulated activity in the fastigial nucleus and ALM (Methods). Unilateral ALM photo-inhibition before movement initiation biased upcoming movements to the ipsilateral direction regardless of a preceding fastigial perturbation (Extended Data Fig 10e-f). Bilateral ALM photo-inhibition during movement initiation led to an increased no lick rate even after a preceding fastigial perturbation (Extend Data Fig 10g). Thus ALM activity drives directional licking despite a fastigial perturbation that destroyed its coding of future movement.

Cerebellar circuitry and roles in motor planning and movement.

The cerebellum is thought to participate in online control of movement [3-6], including eyeblink [7], licking [8] and whisking [8,9]. During eyeblink conditioning, cerebellar nuclei neurons exhibit ramping activity for accurate timing [10,11]. This ramping activity reflects a pause in simple spike activity of the Purkinje cells that controls the eyeblink response [12,13]. Granule cells show rich contextual and anticipatory signals [14,15]. Purkinje cells may combine inputs from the granule cells with teaching signals from the inferior olive to produce appropriately timed pauses in activity during motor planning of tongue movements.

Our results do not preclude a role of the cerebellum in the control of tongue movements. Although our CN perturbations during movement execution did not affect the rate of licking (Fig 1i, l), our study did not examine any potential subtle changes in licking movement kinematics or variabilities induced by the perturbation. Future studies using video-based methods to capture and quantify licking movements in conjunction with cerebellar manipulations will be able to better resolve the role of the cerebellum in licking motor control.

Distributed preparatory activity in the cerebellum and selectively coupled cortico-cerebellar loop

Our results show that the cerebellum is necessary for preparatory activity in frontal cortex. Persistent neural activity is thought to emerge from reverberation of activity mediated by recurrent excitations [16,17]. The cerebellum could not support persistent activity by itself beyond a few hundred milliseconds [18,19]. Persistent activity over seconds [20,21] likely involves interactions with the frontal cortex [19]. Our data is inconsistent with mutual excitation between frontal cortex and the cerebellum. Silencing frontal cortex resulted on average in disinhibition of the CN (Fig 3), inconsistent with the ubiquitous excitatory nature of the projections from the cerebral cortex and pons to the CN, but consistent with a superimposed inhibitory role of Purkinje cells in the cerebellar cortex. ALM may also facilitate CN selectivity by interacting with the olivocerebellar system [22]. Our results are consistent with the CN directly contributing to ALM movement selectivity or providing drive that is necessary for the development of selectivity in ALM.

ALM broadcasts preparatory activity throughout the cerebellum (Extended Data Fig 5-6). However, only the fastigial nucleus influences ALM activity trajectories along the coding direction for planned tongue movements. Whereas the fastigial nucleus output targets the ALM thalamocortical loop, the dentate nucleus is decoupled from this particular loop and does not influence ALM coding of tongue movements and behavior during the task we studied. These data do not exclude a role of the dentate nucleus in other behaviors that involve the corresponding thalamic regions. Different regions of the cerebellum may interact with distinct regions of frontal cortex through different parts of the thalamus [9,23,24]. Our anatomy data also suggest the involvements of other long-range loops. ALM-projecting thalamus received inputs from the SNr [25] and the lateral superior colliculus [26] previously implicated to play roles in controlling licking (Fig 4c, Extended Data Fig 8). The ALM thalamocortical loop thus could be subject to influences of multiple subcortical structures. Our results provide a neural circuit basis for cerebellar control of frontal cortex dynamics during motor planning, opening opportunities for future mechanistic dissections.

Experiment	Mice ID / hemisphere	Figures
CN lesion (wild-type mice)		
Fastigial lesion (n=4)	AN098 (left FN), AN101 (right FN) AN099 (left FN), AN103 (right FN)	Figure 1d-e Extended Data Figure 1a-c
Dentate lesion (n=4)	AN104 (left DN), AN100 (right DN) CD05 (right DN), NL13 (right DN)	Figure 1d Extended Data Figure 1a
CN ChR2 photo-activation (ChR2 virus in wild-type mice)		
Fastigial 1.3s photo-activation (n=6)	AN102 (left FN), AN105 (right FN) AN106 (left FN), AN108 (right FN) AN107 (left FN), AN109 (right FN)	Figure 1h-i, Extended Data Figure 1e-f
Fastigial 0.5s photo-activation (n=6)	AN107 (left FN), AN105 (right FN) AN108 (right FN), AN109 (right FN) NL15 (right FN), CD06 (right FN)	Figure 1g, h
Dentate 1.3s photo-activation (n=8)	AN105 (left DN), AN102 (right DN) AN108 (left DN), AN106 (right DN) AN109 (left DN), AN107 (right DN) NL14 (right DN), CD05 (right DN)	Figure 1g, Extended Data Figure 1e-f
CN photo-inhibition (L7-cre x Ai32 mice)		
0.5s photo-inhibition (n=7)	CD17 (left CN), CD08 (right CN) CD14 (left CN), CD11 (right CN) CD16 (left CN), CD12 (right CN) CD13 (right CN)	Figure 1k, Extended Data Figure 2h-i
1.3s photo-inhibition (n=3)	CD12 (right CN) CD13 (right CN) CD16 (left CN)	Figure 1k-l, Extended Data Figure 2g
CN recording		
CN recording, wild-type mice (n=10)	AN344652 (left CN), CD01 (left CN) AN344651 (left CN), DJ01 (left CN) AN344653 (left CN), NL02 (left CN) AN350831 (left CN), NL01 (left CN) AN350830 (left CN), CD02 (left CN)	Figure 2b, d Extended Data Figure 3, 4
CN recording during ALM photo-inhibition, VGAT-ChR2-EYFP mice (n=8)	NL04 (left CN), NL03 (left CN) NL07 (left CN), NL08 (left CN) NL06 (left CN), NL11 (left CN) NL09 (left CN), NL10 (left CN)	Figure 2b, d, 3a-c Extended Data Figure 5a-d, 6
ALM recording		
ALM recording during CN photo-inhibition, L7-cre x Ai32 mice (n=4)	CD08 (left ALM, right CN) CD12 (left ALM, right CN) CD13 (left ALM, right CN) CD14 (right ALM, left CN)	Figure 3d-f Extended Data Figure 5e
ALM recording during fastigial photo-activation, ChR2 virus in wild-type mice (n=6)	CD06 (left ALM, right FN) NL15 (left ALM, right FN) AN105 (left ALM, right FN) AN107 (right ALM, left FN) AN108 (left ALM, right FN) AN109 (left ALM, right FN)	Figure 2a, c, 4e-f, i, Extended Data Figure 4, 9, 10d
ALM recording during dentate photo-activation, ChR2 virus in wild-type mice (n=3)	NL12 (left ALM, right DN) NL14 (left ALM, right DN) AN106 (left ALM, right DN)	Figure 2a, c, 4g, i Extended Data Figure 4, 9
ALM photo-inhibition + Fastigial perturbation (VGAT-ChR2-EYFP)		
Unilateral ALM photo-inhibition, fastigial perturbation (n=4)	ZD02 (left ALM, right ALM; left FN) ZD03 (left ALM, right ALM; right FN) ZD04 (left ALM, right ALM; left FN) ZD05 (left ALM, right ALM; left FN)	Extended Data Figure 10e-f
Bilateral ALM photo-inhibition, fastigial perturbation (n=3)	ZD02 (bilateral ALM; left FN) ZD03 (bilateral ALM; right FN) ZD04 (bilateral ALM; left FN)	Extended Data Figure 10g

Supplemental Table 1. List of mice and manipulated hemispheres in individual experiments.

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