A cortico–cerebellar loop for motor planning

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Persistent and ramping neural activity in the frontal cortex anticipates specific movements1–6. Preparatory activity is distributed across several brain regions7–9, but it is unclear which brain areas are involved and how this activity is mediated by multiregional interactions. The cerebellum is thought to be primarily involved in the short-timescale control of movement10–12; however, roles for this structure in cognitive processes have also been proposed13–16. In humans, cerebellar damage can cause defects in planning and working memory13. Here we show that persistent representation of information in the frontal cortex during motor planning is dependent on the cerebellum. Mice performed a sensory discrimination task in which they used short-term memory to plan a future directional movement. A transient perturbation in the medial deep cerebellar nucleus (fastigial nucleus) disrupted subsequent correct movements without hampering movement execution. Preparatory activity was observed in both the frontal cortex and the cerebellar nuclei, seconds before the onset of movement. The silencing of frontal cortex activity abolished preparatory activity in the cerebellar nuclei, and fastigial activity was necessary to maintain cortical preparatory activity. Fastigial output selectively targeted the behaviourally relevant part of the frontal cortex through the thalamus, thus closing a cortico-cerebellar loop. Our results support the view that persistent neural dynamics during motor planning is maintained by neural circuits that span multiple brain regions7,8, and that cerebellar computations extend beyond online motor control13–15,18.

Head-fixed mice were presented with a pole during a sample period of 1.3 s, and discriminated its location (anterior or posterior) using their whiskers (Fig. 1a). The mice held their responses in short-term memory and planned for the upcoming movement during a delay period of 1.3 s. After this, an auditory ‘go’ cue (0.1 s) signalled the response period and mice reported the location of the pole by skilled directional licking (left or right) to obtain a water reward.

During such a delay period, it is known that preparatory activity in the anterior lateral motor cortex (ALM; Fig. 1b) is critical for subsequent correct movements19–22. ALM neurons exhibit persistent and ramping activity that predicts specific future movements, long before the onset of movement.

The ALM projects to the cerebellum via the basal pontine nucleus23,24. We tested the involvement of the cerebellum in the delayed response task by lesioning either the fastigial nucleus or the dentate nucleus (Fig. 1c, Methods). We separately examined behavioural choice (the direction of the first lick after the ‘go’ cue) and movement execution (licking duration and frequency). Fastigial lesions impaired the initiation of contralesional licking (Fig. 1d) and delayed the initiation of contralesional licking following the ‘go’ cue (Fig. 1e). After the initiation of movement, licking duration and frequency were unaffected (Fig. 1e, Extended Data Fig. 1b, c). Dentate lesions (around 1.5 mm away from the fastigial nucleus) did not change behavioural choice and movement execution (Fig. 1d).

We tested the involvement of the fastigial nucleus during specific behavioural periods by disrupting activity using channelrhodopsin-2 (ChR2) activation (photoactivation, Fig. 1f, Methods). Fastigial photoactivation during the early sample period had no effect on behavioural choice ($P > 0.05$ for either trial type, bootstrap; Methods). Photoactivation during the delay and response periods both resulted in incorrect choices (Fig. 1g, h), which were induced even when the photostimuli ceased 800 ms before the movement. Photoactivation during the delay period biased future movements to either the contralateral or ipsilateral direction in individual mice (Fig. 1h, Extended Data Fig. 1e).

Photoactivation immediately before the onset of movement (response period) produced a contralateral bias (Fig. 1i), which is opposed to the directional bias induced by fastigial lesions. The distinct patterns of bias induced by photoactivation in the delay and in the response periods suggest temporally specific roles for the fastigial nucleus in driving movement planning and initiation.

Fastigial photoactivation during the response period did not block or perturb the frequency and duration of licking movements (Fig. 1i). In addition, photoactivation during the delay period did not evoke early licks (Extended Data Fig. 1f). These data show that rhythmic licking is independent of fastigial activity, but they do not rule out the involvement of the fastigial nucleus in controlling the direction and timing of licking bouts via its descending input to oscillators in the medulla that drive licking25. Dentate photoactivation produced little effect on behavioural choice (Fig. 1g, $P = 0.06$, bootstrap; Extended Data Fig. 1e), which is consistent with results from the lesion experiments.

Next, we silenced fastigial activity to test its necessity for correct behavioural choice. We photostimulated ChR2-expressing Purkinje cells, which inhibited the activity of the cerebellar nucleus (CN) (photoinhibition, Fig. 1j, Extended Data Fig. 2, Methods). Photoinhibition during the early sample period did not affect behavioural choice (Fig. 1k, $P > 0.05$ for either trial type, bootstrap; Methods). Photoinhibition during the delay and response periods resulted in incorrect behavioural choices, with a larger effect induced in the delay period (Fig. 1k, Extended Data Fig. 2g, h). Photoinhibition during the delay period biased future movements to either the contralateral or the ipsilateral direction in individual mice (Extended Data Fig. 2g, h). Photoinhibition during movement initiation (response period) produced an ipsilateral bias (Fig. 1k), consistent with that observed after fastigial lesions, and opposite from the bias induced by fastigial photoinactivation. Photoinhibition minimally affected movement execution (Fig. 11), and no other movements were consistently evoked during photoinhibition (Methods, Extended Data Fig. 2e, f, i). These results reveal that fastigial activity, starting from the late sample period and continuing throughout the delay period, is required for correct behavioural choice.

We compared the activity in the ALM and the CN by recording from single units (ALM, $n = 1,194$ units; CN, $n = 564$ units; Extended Data Fig. 3, Methods). Most ALM pyramidal neurons distinguished ‘lick-left’ and ‘lick-right’ trials in spike counts (980/1,194, $P < 0.05$, two-tailed t-test, Methods). A similar proportion of CN neurons showed trial-type selectivity (416/564). Individual ALM and CN neurons exhibited diverse activity patterns, including selective persistent activity and ramping activity during the delay period (Fig. 2a, b). Preparatory activity (selectivity before the response period) was more prevalent in ALM (ALM, 59% of neurons; CN, 35% of neurons; $P < 0.001$, $\chi^2$ test).

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Peri-movement activity (selectivity during the response period) was equally prevalent in both regions (ALM, 67%; CN, 66%). In both regions, a similar proportion (less than 10%) of neurons showed modulated spike rates during individual licks (Extended Data Fig. 4). Selectivity for lick-left and lick-right trials emerged gradually in both ALM and CN population averages (Fig. 2c, d), starting during the sample period and reaching a maximum during the response period. An ‘error’ trial was recorded if the mouse licked in the opposite direction to the instruction provided by the object location. A small group of CN neurons signalled the location of the object; however, most CN neurons predicted the upcoming lick direction (67%; Extended Data Fig. 4), which is similar to findings in the ALM.** Thus, CN selectivity during the delay period is related to future movement, and is characteristic of preparatory activity that instructs subsequent movement.

Preparatory activity was present in all three cerebellar nuclei (Extended Data Figs. 3, 4). In the fastigial nucleus, distinct patterns of selectivity were observed during the early compared with the late delay period, in which equal numbers of neurons preferred either contralateral or ipsilateral movement during the early delay. In addition, a population-level preference for contralateral movement developed just before movement initiation** (Extended Data Fig. 4g), which is consistent with the directional biases induced by fastigial lesion and optogenetic manipulations during movement initiation (Fig. 1). These results show that there is widespread activity related to motor planning and movement initiation in the CN.

The cerebellum is thought to process information over timescales of hundreds of milliseconds. Cerebellar preparatory activity lasting seconds could be inherited from the ALM. We recorded from the CN while bilaterally photoinhibiting the ALM during the delay period (Fig. 3a, Methods). The activity of many CN neurons was rapidly altered by ALM photoinhibition (185/389, P < 0.01, two-tailed t-test; latency, 10.7 ± 4.0 ms, mean ± s.e.m.), including that of neurons with selective persistent and ramping activity (Extended Data Fig. 5a). Photoinhibition of the ALM caused a greater proportion of CN neurons to increase in activity, resulting in a net disinhibition of the CN (Fig. 3b, 123/185 increase, 62/185 decrease, P < 0.001, binomial test). The selectivity of the CN for lick-left and lick-right trials was abolished by photoinhibition of the ALM (Fig. 3c, Extended Data Fig. 5b–d).

Photoinhibition of either side of the ALM reduced preparatory activity in the CN (Extended Data Figs. 6a–d), whereas photoinhibition of motor cortex regions posterior to the ALM had a lesser effect on CN preparatory activity (Extended Data Fig. 6e). Thus, the ALM seems to provide widespread input to the cerebellum to drive CN preparatory activity during motor planning.

We tested whether ALM preparatory activity depends on CN activity by recording from ALM while photoinhibiting the CN during the delay period (Fig. 3d, Methods). The activity of many ALM neurons was rapidly altered by CN photoinhibition (197/454, P < 0.01, two-tailed t-test, Extended Data Fig. 5e, latency, 7.1 ± 3.8 ms). However, the average spike rate was nearly unchanged (control, 4.15 spikes per second; photoinhibition, 4.39 spikes per second; P = 0.58, two-tailed t-test). The responses of individual ALM neurons increased (108/197) or decreased (89/197) during CN photoinhibition (Fig. 3e). ALM trial-type selectivity was abolished by CN photoinhibition (Fig. 3f), similar to observations after partial inactivation of the thalamus.** Thus, CN activity is necessary for maintaining cortical selectivity for planned movement, and preparatory activity is maintained in a cortico-cerebellar loop.

Preparatory activity requires reciprocal excitation between the ALM and the thalamus.** We mapped connectivity between the CN,
the thalamus and the ALM using retrograde and anterograde tracers in the ALM and the CN (Methods, Fig. 4a, Extended Data Figs. 7, 8). Fastigial projections mostly overlapped with parts of the thalamus that project to the ALM (that is, the ventral medial nucleus and parts of the ventral anterior-lateral nucleus, whereas dentate projections targeted an adjacent region that was more dorsal and lateral (that is, primarily the ventral anterior-lateral nucleus) (Fig. 4b, c).

We investigated the influence of the CN on the ALM thalamocortical loop during motor planning. We perturbed fastigial or dentate activity using ChR2 activation during the early delay period while monitoring ALM preparatory activity (Fig. 4d). Fastigial photoactivation induced rapid changes in ALM activity (latency, $4.5 \pm 2.6$ ms; Extended Data Fig. 9a), which is consistent with a disynaptic pathway through the thalamus. Selective persistent and ramping activity was abolished in most ALM neurons (Fig. 4e, top). In a minority of neurons, photoactivation created new preparatory activity (Fig. 4e, bottom). Transient photoactivation of the fastigial nucleus altered ALM preparatory activity long (more than 1 s) after cessation of the photostimulus (Fig. 4f, Extended Data Fig. 9b). Dentate photoactivation also induced changes in the ALM response (Fig. 4g; latency, $8.3 \pm 5.0$ ms).

Next, we differentiated between the effects of fastigial and dentate photoactivation on the preparatory activity of the ALM. We analysed population dynamics in ‘activity space’, where each dimension corresponds to the activity of one neuron.$^{6,20,29}$ We estimated a ‘coding direction’ (cd) in activity space, along which preparatory activity maximally discriminated upcoming licking directions (Fig. 4h, Methods). In control trials, activity trajectories from lick-left and lick-right trials diverged from each other along the cd during the sample period and remained separated throughout the delay period, providing a stable neural substrate for a short-term memory of future movement$^{20}$ (Fig. 4i). Fastigial photoactivation resulted in a collapse of activity trajectories. Additional experiments showed that fastigial photoactivation abolished the relationship between activity trajectories and preparatory activity long (more than 1 s) after cessation of the photostimulus (Fig. 4f, Extended Data Fig. 9b). Dentate photoactivation also induced changes in the ALM response (Fig. 4g; latency, $8.3 \pm 5.0$ ms). Notably, activity quickly recovered after dentate photoactivation (Fig. 4g).
specific future movements (Supplementary Information, Extended Data Fig. 10). By contrast, dentate photoactivation affected activity trajectories along the cd only minimally, despite having an effect on ALM neuron responses (Fig. 4i, Extended Data Fig. 9c). Therefore, fastigial photoactivation eradicated the coding of future movement, whereas dentate photoactivation did not.

Our results show that the cerebellum is critical for the coding of future movement in the frontal cortex. Considering its roles in the timing of movement, the cerebellum may provide an urgency drive that is necessary for the emergence of selectivity in the ALM thalamo-cortical loop in the preparation of movement, which is consistent with attractor models of motor planning.

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METHODS

Mice. This study is based on data from 70 mice (age >60 weeks where P is postnatal day; both male and female mice, Supplementary Table 1). Thirteen C57Bl/6 mice were used for CN ChR2 photoactivation experiments. A subset of these (10 mice) was used for ALM recordings during photoactivation of the CN. Another subset (2 mice) and six additional C57Bl/6 mice were used for CN lesion experiments (6 mice). Seven L7-cre 

Ai32 (Rosa26-LSL-Chr2-eYFP, JAX Stock 012639) mice were used for CN photoactivation experiments in the delayed response task. A subset of these mice (4 mice) was used for ALM recordings during CN photoinhibition. Two additional L7-cre × Ai32 mice were used to characterize CN photoinhibition using electrophysiology. Six additional L7-cre × Ai32 mice (not listed in Supplementary Table 1) were used to quantify the general effects of CN photoinhibition on movements. Ten C57Bl/6 mice were used for CN recording experiments. Eight VGAT-ChR2-eYFP mice (Jackson Laboratory; JAX Stock 014548) were used for CN recordings during ALM photoactivation. Four VGAT-ChR2-eYFP mice were used for behavioural experiments in which both ALM and CN activity were manipulated to test the causal role of ALM activity in behaviour following a fastigial perturbation. Twelve C57Bl/6 mice (not listed in Supplementary Table 1) were used for anatomical tracing.

All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees at Baylor College of Medicine, Janelia Research Campus and the Erasmus Medical Center. Mice were housed in a 12:12 reverse light/dark cycle and tested during the dark phase. On days when they were not tested, mice received 0.5–1 ml of water. On other days, mice were tested in experimental sessions lasting 1–2 h, during which they received all their water (0.3 to 2 ml). If mice did not maintain a stable body weight, they received supplementary water. All surgical procedures were carried out aseptically under 1–2% isoflurane anaesthesia. Buprenorphine HCl (0.1 mg kg⁻¹) or sustained-release meloxicam (4 mg kg⁻¹) was used for pre- and post-operative analgesia. Ketoprofen (5 mg kg⁻¹) was used at the time of surgery and post-operatively to reduce inflammation. After the surgery, mice were allowed to recover for at least three days with free access to water before water restriction.

Surgery. Mice were prepared for photoactivation and electrophysiology with a clear-skull cap and a headpost. The scalp and peristium over the dorsal surface of the skull were removed. A layer of cyanoacrylate adhesive (Krazy Glue, Elmer's Products) was directly applied to the intact skull. A custom-made headpost was placed on the skull (approximately over the visual cortex) and cemented in place with clear dental acrylic (Lang Dental Jett Repair Acrylic; 1223-clear). A thin layer of clear dental acrylic was applied over the cyanoacrylate adhesive covering the entire exposed skull, followed by a thin layer of clear nail polish (Electro-Microscopy Sciences, 72180).

In wild-type mice prepared for CN ChR2 photoactivation experiments, 150 nl of AAV2-hSyn1-(h134R)ChR2-eYFP virus (UNC viral core, titre 5.7 × 10⁷ vector genomes) was injected in the fastigial or dentate nucleus through the implanted optical fibre for 5–10 min while the mice were under 2% isoflurane anaesthesia. Light-induced heating produced a localized lesion around the fibre tip. The lesion areas were estimated post hoc, by measuring the size of areas in which neuron labelling was absent (anti-NeuN staining, Millipore ABN78, 1:1,000; Fig. 1c, Extended Data Fig. 1a). Lesion sizes were between 602–878 µm, close to the diameters of the fastigial nucleus or the dentate nucleus. The mice were tested on subsequent days. See Supplementary Table 1 for the list of mice and lesioned hemispheres.

CN ChR2 photoactivation. For ChR2 photoactivation of the CN, 13 wild-type mice injected with AAV2-ChR2-eYFP virus (UNC viral core, titre 5.7 × 10⁷ vector genomes) were used. Light was delivered to the CN through an optical fibre. In six mice, both the fastigial and the dentate nucleus on opposite hemispheres were tested: left fastigial/right dentate nucleus was tested in 3 mice and right fastigial/left dentate nucleus was tested in the other three mice. In those mice, the fastigial and the dentate nucleus were tested on separate days. In another three mice, only the right fastigial nucleus was tested. In one four mice, only the right dentate nucleus was tested. In one of the fastigial-stimulation mice, the injection of ChR2 virus missed the CN and its data were excluded. See Supplementary Table 1 for the list of mice and manipulated hemispheres in individual experiments.

Light from a 473-nm laser (Laser Quantum, Gem 473) was controlled by an acousto-optical modulator (Quanta Tech) and a shutter (Vincent Associates). To prevent the mice from distinguishing photoactivation trials from control trials using visual cues, a ‘masking flash’ was delivered using 470 nm LEDs (Laxxon Star) near to the eyes of the mice. The masking flash began as the pole started to move and continued through the end of the period in which photoactivation could occur. Photoactivation was deployed on 25% of the behavioural trials. During ALM recordings, photoactivation was deployed on 33% of the trials. The photostimulus was a pulse of light (5–ms pulse duration) delivered at 20 Hz and a range of peak powers (5, 10 and 15 mW). The power values reported in the paper indicate average powers (0.5, 1 and 1.5 mW). The powers were measured at the fibre tip. The photostimulus started at the beginning of a task period and continued for either 0.455 s (10 pulses) or 1.255 s (26 pulses).

We performed photoactivation both during the early parts of the sample and delay period (0.455 s; Fig. 1g, h) and throughout the entire period (1.255 s; Extended Data Fig. 1e). Our primary experiments were centred around sub-period manipulations (early sample and early delay). This experimental design was chosen for two reasons. First, sub-period manipulations sought to better resolve specific involvements of the CN in time. Manipulations during the entire sample period (1.3 s) may affect the beginning of the motor planning process, and manipulations during the entire delay period up to the ‘go’ cue may interfere with movement initiation. Second, we sought to use the early delay period manipulation to examine any persistent effect on preparatory activity (Fig. 4). If a brain region is involved in maintaining preparatory activity, transiently disrupting it during the early delay should subsequently disrupt preparatory activity. We later tested manipulations that spanned the entire behavioural periods and included the response period to examine if the CN is involved in preparatory activity (Fig. 5). We also investigated if the CN may affect the beginning of the motor planning process, and manipulations during the entire delay period up to the ‘go’ cue may interfere with movement initiation.

We performed photostimulation both during the early parts of the sample and delay period (0.455 s; Fig. 1g, h) and throughout the entire period (1.255 s; Extended Data Fig. 1e). Our primary experiments were centred around sub-period manipulations (early sample and early delay). This experimental design was chosen for two reasons. First, sub-period manipulations sought to better resolve specific involvements of the CN in time. Manipulations during the entire sample period (1.3 s) may affect the beginning of the motor planning process, and manipulations during the entire delay period up to the ‘go’ cue may interfere with movement initiation. Second, we sought to use the early delay period manipulation to examine any persistent effect on preparatory activity (Fig. 4). If a brain region is involved in maintaining preparatory activity, transiently disrupting it during the early delay should subsequently disrupt preparatory activity. We later tested manipulations that spanned the entire behavioural periods and included the response period to examine if the CN is involved in preparatory activity (Fig. 5). We also investigated if the CN may affect the beginning of the motor planning process, and manipulations during the entire delay period up to the ‘go’ cue may interfere with movement initiation.

For CN photoinhibition both during sub-periods, we tested L7-cre × Ai32 mice. Photostimulation of Purkinje cells was carried out by directing a blue laser (beam diameter 400 µm at 4σ) to the brain surface through a cranial window. The photostimulus was a 40 Hz sinusoid (average power, 4.5 mW) lasting for 1.3 s, including a 100–200 ms linear ramp during the laser offset to reduce rebound neuronal activity. Photostimulation was deployed on 25% of the behavioural trials. During ALM recordings, photostimulation was deployed on 33% of the trials. A masking flash was delivered on all trials. Photostimulation was performed at two different locations: 3 mm posterior and 2 mm lateral from lambda (the cerebellar cortex); and 1 mm posterior and 2 mm lateral to lambda (the inferior colliculus). Photostimulation of the cerebellar cortex silenced fastigial activity whereas photostimulation of the inferior colliculus did not (Extended Data Fig. 2).

For photoinhibition of the CN during behavioural trials, we tested L7-cre × Ai32 mice. Photostimulation of Purkinje cells was carried out by directing the blue laser to the brain surface through a cranial window. We manipulated the left hemisphere in three mice and the right hemisphere in the other four mice. The photostimulus (average power 0.5, 1.5 or 4.5 mW) started at the beginning of a task period and continued for either 0.5 or 1.3 s, including the 100–200 ms linear ramp.

We performed CN photoinhibition both during parts of the behavioural trial period (0.5 s; Fig. 1k) and over the entire period (1.3 s; Extended Data Fig. 2g), for the same reasons described in ‘CN ChR2 photoactivation’. In addition to early delay period manipulations, we also investigated early and late delay photoinhibitions to resolve CN involvements during specific sub-periods of the delayed response task (Extended Data Fig. 2h). Seven mice were initially tested for sub-period photoinhibitions. Later, three of the seven mice were tested...
with photoinhibition over the entire behaviour periods to confirm the directional bias induced by response period photoinhibition (Extended Data Fig. 2g, Supplementary Table 1).

In four L7-cre × Ai32 mice (Supplementary Table 1), we recorded from the ALM during contralateral CN photoinhibition. Photoinhibition (average power, 1.5 or 4.5 mW) started at the beginning of the delay period and continued for 1.3 s, including the 100–200 ms linear ramp. CN photoinhibition and effects on movement. In six naive L7-cre × Ai32 mice, we examined the general effects of CN photoinhibition on movements. One optical fibre was implanted in the fastigial nucleus and another fibre was implanted in the dentate nucleus in the opposite hemisphere to photostimulate Purkinje cell axons in the CN. We tested a range of laser powers (average power, 1.5, 5, 10 and 20 mW), including the photostimulus intensities used to photoinhibit CN activity in the delayed response task (1.5 mW). The photostimulus was pulses of light (5–10 ms pulse duration, 20–40 Hz, 15–50 mW peak powers). Videos (Point Grey, CM3-US-13T3M-CS or Basler, ac640-300gm) were recorded while mice underwent photoinhibition either in the head-fixed configuration or unrestrained. In head-fixed mice (n = 6), jaw movement and eyelash were tracked using custom software. In unrestrained mice (n = 2), the effect of photoinhibition on movements and postures were manually scored on a scale of 0 to 4 that rated dystonia-like movements: 0, no motor abnormalities; 1, slightly slowed or abnormal motor behaviour; 2, mild impairment, mild and transient dystonic postures, weak tremor; 3, moderate impairment, dystonic postures, cannot balance the body, major tremor; 4, severe impairment, sustained dystonic postures and limited movements. Two individuals scored the videos independently and the scores were averaged. These were used to conduct the experiments.

In head-fixed, high-intensity photoinhibition (20 mW) of the dentate nucleus produced jaw movements (Extended Data Fig. 2f). No tongue protrusion was observed in all photoinhibition conditions. No consistent eyelash was evoked. At the photoinhibition intensities used in the delayed response task (1.5–5 mW), no movement was consistently evoked.

In unrestrained mice, high-intensity photoinhibition (20 mW) of both the fastigial nucleus and the dentate nucleus produced dystonia-like movements and posture changes. This included loss of balance and extension of the contralateral limbs (Extended Data Fig. 2e). However, at the photoinhibition intensities used in the delayed response task (1.5–5 mW), no consistent movement was detected (Extended Data Fig. 2e).

**ALM photoinhibition.** The ALM is centred on bregma anterior 2.5 mm, lateral 1.5 mm [19–21]. For photoinhibition of the ALM, we photostimulated cortical GABAergic (γ-aminobutyric acid) neurons in VGAT-ChR2-eYFP mice (8 mice). Photostimulation was performed through the clear-skull cap implant by directing the blue laser over the skull (beam diameter, 400 µm at 4°, bregma anterior 2.5 mm, lateral 1.5 mm). The light transmission through the intact skull was 50% [19]. Photostimulation was delivered on 25% of the behavioural trials. During CN recordings, photoinhibition was deployed on 33% of the trials. A masking flash was delivered on all trials.

We photoinhibited ALM for 1.3 s at the beginning of the delay period, including a 100 ms linear ramp at the laser offset to minimize rebound excitation. This photostimulus was empirically determined to produce robust photoinhibition in ALM [20,21]. The photoinhibition silenced 90% of spikes in a cortical area of 1 mm radius (at half-maximum) through all cortical layers. For unilateral ALM photoinhibition (Extended Data Figs. 6, 10), we used a 40 Hz sinusoidal photostimulus (1.5 mW average power at the skull surface) at 2.5 mm anterior and 1.5 mm lateral from bregma. For bilateral ALM photoinhibition (Fig. 3, Extended Data Figs. 5, 6, 10), we used a constant photostimulus and a scanning galvo (GVSM002, Thorlabs), which stepped the laser beam sequentially through the photoinhibition sites at the rate of 1 step per 5 ms (step time, 0.2 ms; dwell time, 4.8 ms; measured using a photodiode). Eight photoinhibition sites covered the ALM on both hemispheres. On each hemisphere, four photoinhibition sites were spaced in 1 mm spacing, at anterior 2–3 mm and lateral 1–2 mm from bregma. Peak power was adjusted based on the number of photoinhibition sites to achieve an average power of 1.5 mW per site. ALM photoinhibition after a fastigial perturbation. To test the causal role of ALM activity in driving directional licking after a fastigial perturbation, we tested four VGAT-ChR2-eYFP mice (Extended Data Fig. 10). We confirmed that ChR2 was expressed in Purkinje cells as well as other GABAergic neurons in the cerebellum [22]. Photostimulation (40 Hz sinusoid; average power, 1.5 mW) was delivered to the fastigial nucleus through an optical fibre to perturb CN activity. Photostimulation was started at the onset of the delay period and ended at the onset of the ‘go’ cue (500 ms in duration). Unilateral ALM photoinhibition before the ‘go’ cue was previously found to bias the upcoming licking to the ipsilateral direction [20]. We confirmed the ipsilateral bias here (Extended Data Fig. 6b, 10e). In three VGAT-ChR2-eYFP mice, we also tested bilateral ALM photoinhibition. Bilateral photostimulation started at the onset of the ‘go’ cue and lasted for 1.3 s. On randomly interleaved trials, we either perturbed the fastigial nucleus, or photoinhibited ALM (unilateral or bilateral), or perturbed the fastigial nucleus followed by ALM photoinhibition (Extended Data Fig. 10f, g). Photostimulation was deployed on 25% of the behavioural trials. A masking flash was delivered on all trials.

**Behavioural data analysis.** Performance was computed as the fraction of correct choices, excluding lick early trials and no lick trials (Fig. 1g, Extended Data Figs. 1e, 2g, 10b). Chance performance was 50%. We also separately computed the performance for lick-right and lick-left trials (Fig. 1, Extended Data Figs. 1, 2, 6, 10). The significance of the change in performance in each photostimulation condition was determined using bootstrap to account for variability across mice, sessions and trials. We tested against the null hypothesis that the change in performance caused by photostimulation was due to normal behavioural variability. In each round of bootstrap, we replaced the original behavioural dataset with a resampled dataset in which trials were shuffled with replacement from: (1) mice, (2) sessions performed by each mouse, (3) trials within each session. We then computed the performance change on the resampled dataset. Repeating this procedure 10,000 times produced a distribution of performance changes that reflected the behavioural variability. The P value of the observed change in performance was computed as the fraction of the times the bootstrap produced an inconsistent change in performance (for example, if a performance decrease was observed during photostimulation, the P value was the fraction of times a performance increase was observed during bootstrap, one-tailed test).

**Electrophysiology.** Extracellular spikes were recorded using 32-channel NeuroNexus silicon probes (A4×8-5mm-100-200-177) or 64-channel Cambridge NeuroTech silicon probes (H2 acute probe, 25 µm spacing, 2 shanks). The 32-channel voltage signals were multiplexed, digitized by a PCi6133 board at 400 kHz (National Instruments) at 14 bit, demultiplexed (sampling at 25,000 Hz) and stored for offline analysis. The 64-channel voltage signals were amplified and digitized on an Intan RHD2164 64-Channel Amplifier Board (Intan Technology) at 16 bit, recorded on an Intan RHD2000-Series Amplifier Evaluation System (sampling at 20,000 Hz) using Open-Source RHD2000 Interface Software from Intan Technologies. Data were stored in two formats; for offline analysis.

For ALM recordings, a small craniotomy (diameter, < 1 mm) was made one day before the recording session (2.5 mm anterior, 1.5 mm lateral from bregma) [23]. A silicon probe was acutely inserted 0.9–1.11 mm below the brain surface before the start of each recording session. To minimize brain movement, a drop of silicone gel (3–4680, Dow Corning) was applied over the craniotomy after the electrode was in the tissue. The tissue was allowed to settle for several minutes before the recording started. Two to six recordings were made from each craniotomy. In ten mice, recordings were from the left ALM. In two mice, recordings were from the right ALM. ALM recordings were always contralateral to the side of CN manipulations (Supplementary Table 1).

For CN recordings, two craniotomies (diameter, 1.5 mm) were made successively over the left cerebellum to target the CN (first at 2.5 mm posterior, 1.5 mm lateral from lambda; then at 2 mm posterior, 3 mm lateral from lambda). After a craniotomy was opened, recordings were made at different locations within the craniotomy over the next 5–7 days. A silicon probe was acutely inserted 2.1–2.4 mm below the brain surface before the start of each session. On the last recording session, the silicon probe was painted with DiI to label the recording track. All the recording locations within a craniotomy were reconstructed post-hoc relative to the labelled track (Extended Data Fig. 3). Reconstructions were based on the insertion locations on the brain surface. Recording depths were inferred from manipulator readings and verified on the basis of histology. The second craniotomy was opened after the first craniotomy had been sampled.

**Electrophysiology data analysis.** The extracellular recording traces were band-pass-filtered (300–6 kHz). Events that exceeded an amplitude threshold (four standard deviations of the background) were subjected to manual spike sorting to extract single units [24].

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>0.45 ms as putative pyramidal neurons (1,194/1,309). Units with intermediate values (0.35–0.45 ms, 33/1,309) were excluded from the analyses. This classification was previously verified by optogenetic tagging of GABAergic neurons. We concentrated our analyses on the putative pyramidal neurons (n = 1,194). Nine of the ten mice were tested with CN ChR2 photoactivation during ALM recordings (Fig. 4, Extended Data Fig. 10; n = 912 putative pyramidal neurons). One mouse was excluded because the injection of ChR2 virus missed the CN. For ALM recordings during CN photoinhibition (Fig. 3, Extended Data Fig. 5), 490 single-units were recorded in 4 mice across 19 sessions. In total, 494/490 units were putative pyramidal neurons.

For CN recordings during behaviour trials, 878 single-units were recorded in 18 mice across 169 sessions (Fig. 2). CN single-units were not classified on the basis of spike shapes. We estimated unit locations on the basis of recording track labelling, recording depth and the laminations of activity patterns across the recording shanks (Extended Data Fig. 3). CN boundaries were visible in DAPI staining (Extended Data Fig. 3). 314 units were from outside of the CN (mostly from the cerebellar cortex). 268 units were from the fastigial nucleus, 139 units were from the interposed nucleus and 157 units were from the dentate nucleus. Eight of the 18 mice were tested with ALM photoinhibition during CN recordings (Fig. 3, Extended Data Figs. 5, 6, n = 389).

To characterize CN photoinhibition in L7-cre × Ai32 mice, 60 single units were recorded in 2 mice across 11 recording sessions (Fig. 1), Extended Data Fig. 2. The recordings were targeted to the left fastigial nucleus. Thirty-four units were from outside of CN (mostly from the cerebellar cortex). Twenty-six units were from the fastigial nucleus. The mice were awake but non-behaving during the recordings. Neurons were tested for significant trial-type selectivity during the sample, delay or response periods, using the spike counts from the lick-left and lick-right trials (two-tailed t-test, P < 0.05). Neurons that significantly differentiated trial types during any one of the periods were deemed ‘selective’. To compute selectivity in Figs. 2, 3 and Extended Data Figs. 4, 5, 6, we first determined the preferred trial type of each neuron using spike counts from a subset of the trials (10 trials for ALM, 20 for CN); selectivity was calculated as the difference in spike rate between lick-left and lick-right trials in the remaining data. Selecting the preferred trial type using either sample, delay or response period data yielded qualitatively similar preparatory activity.

To quantify the effect of photostimulation on individual neuron spike rates (Figs. 3, 4, Extended Data Figs. 5, 9), spike counts from lick-left and lick-right trials were pooled. We used two-tailed t-tests on spike counts binned in various windows (for example, Fig. 3 and Extended Data Fig. 5, the whole delay period; Fig. 4f, 400-ms time bins in steps of 50 ms) to test for a significant change in spike rate between control and photostimulation trials.

Quantification of the effect of photostimulation on selectivity was complicated by the fact that selectivity was coupled to upcoming movements (Extended Data Fig. 5a). To compare correct versus incorrect trials, we used only correct trials to compute selectivity; this would miss the trials in which photostimulation caused the mice to switch future movements, thus underestimating the effect of photostimulation on selectivity. We therefore used all trials (correct and incorrect, grouped by instructed trial types) when quantifying selectivity changes caused by photostimulation (Fig. 3c, f, Extended Data Figs. 5, 6, 9).

Analysis of ALM population dynamics in the activity space. To analyse the relationship between ALM population activity and upcoming movements, we restricted the analysis to the recording sessions with >5 neurons recorded simultaneously for >5 trials per condition (28/39 sessions, 8–46 neurons recorded simultaneously; Fig. 4, Extended Data Fig. 10). For a population of n neurons, we found an n × 1 vector in the n dimensional activity space that maximally separated the response vectors in lick-right and lick-left trials; we term this vector the coding direction (cd).

Average spike counts were computed in 400-ms windows in 10-ms steps. For each movement direction (lick-right and lick-left) we computed the average spike counts \( \mu_{\text{lick-right}} \) and \( \mu_{\text{lick-left}} \), n × 1 response vectors that described the population response at each time point, t. We computed the difference in the mean response vectors, \( \Delta \mu = \mu_{\text{lick-right}} - \mu_{\text{lick-left}} \). We also computed the r.m.s. of the difference in spike rate between lick-right and lick-left trials, and \( \Delta \mu_{\text{r.m.s.}} = \sqrt{\sum_{t=1}^{N} \Delta \mu_{t}^2} \). The probability of the mouse licking right in photostimulation trials was calculated as a function of projected responses, \( \Delta \mu \) (Extended Data Fig. 10c, d). Data from multiple sessions were pooled.

Anatomy tracing. We mapped connectivity between the CN, thalamus, ALM, sub-striatum nigra reticulata (SNr) and the lateral superior colliculus. The lateral superior colliculus was previously implicated to have roles in controlling licking (Fig. 4a–c, Extended Data Figs. 7, 8). Mice were anaesthetized using isoflourane and fixated on a high precision stereotaxic frame (David Kopf or Stoelting). Coordinates used to target brain areas of interest used either bregma or lambda as the reference points. The coordinates were (in mm): ALM: bregma anterior 2.5, lateral 1.5, depth 0.8; SNr: lambda anterior 1.1, lateral 1.4, depth 4.5; superior colliculus: lambda anterior 0.9, lateral 2.3, dorsal superior colliculus: lambda posterior 2.5, lateral 2.5, depth 2.4. To label the ALM-projecting thalamic, 10% biotin dextran amine BDA-10k (Sigma), or 1% Cholera toxin subunit b (Ctb) conjugated to Alexa647 (Invitrogen) dissolved in PBS were iontophoretically injected. Glass pipettes with a tip size of 20 µm were filled with either BDA or CIB and 4 mA of positive current was applied with a 7-s duty cycle for 15 min. Viral vector-assisted tracing was applied to the other brain regions. AAV2-CAG-eGFP or AAV2-CAG-IRFP (100–150 µl; UNC viral core, titres 1.0–1.2 × 1013 vg ml⁻1) were pressure-injected in the target areas. After each injection, the pipette was left in place for >10 min before being slowly withdrawn. Brains were processed for immunohistochemistry 10 days after the injection. Mice were deeply anaesthetized with an overdose of Nembutal (0.2 ml, i.p.) and transcardially perfused with 20 ml saline followed by 50 ml 4% paraformaldehyde (PFA) in PBS. Brains were extracted and post-fixed in 4% PFA for 2 h and incubated in 10% sucrose overnight at 4°C. Brains were then embedded in gelatin and cryoprotected in 30% sucrose in PB, frozen on dry ice, and sectioned using a freezing microtome (50 µm thick). For immunohistochemistry, sections were blocked for 1 h at room temperature in PBS with 0.4% Triton X-100 and 10% N-hydroxysuccinimide (Sigma) and 1% BSA in a mixture of primary and secondary antibodies. Axonal projections and neurons were visualized using: mouse anti-NeuN (1:1,000, Millipore), chicken anti-GFP (1:2,000, Aves), rabbit anti-RFP (1:2,000, Rockland), mouse anti-vGlut2 (1:1,000, Millipore) and streptavidin–Alexa647 (1:400, Jackson). Slices were counterstained with DAPI (1:100,000, Invitrogen) and mounted with mounting medium for fluorescence (Mowiol, Sigma). Stacks of images were acquired on an upright LSM 700 confocal microscope (Carl Zeiss) operated with Zeiss Zen Software (v.2.2) and post-hoc adjusted and processed in FIJI software (v.1.46) with appropriate plugins ([https://imagej.net/Fiji](https://imagej.net/Fiji)).

Anatomy analysis. We aligned each coronal section to the Allen Mouse Common Coordinate Framework (CCF) using landmark-based image registration. The registration target was the 10 µm per voxel CCF anatomical template. To align a coronal section, we first manually selected the coronal plane in the anatomical template that best corresponded to the section. Next, we manually placed control points at corresponding local landmarks in each image (Extended Data Fig. 7a). Thirty to fifty control points were placed in a single image. All control points were outside of the thalamus. Next, the image was warped to the CCF using an affine transformation followed by a non-rigid transformation using b-splines. Images were warped using the B-spline Grid, Image and Point based Registration package available on the Mathlab FileExchange ([https://www.mathworks.com/matlabcentral/fileexchange/20057-b-spline-grid–image-and-point-based-registration](https://www.mathworks.com/matlabcentral/fileexchange/20057-b-spline-grid–image-and-point-based-registration)). We performed this procedure independently for each brain section. Two to six sections at the rostro-caudal level of the thalamus were analysed (bregma posterior 1.1–2.1 mm in the CCF).

We quantified the connectivity of the fastigial nucleus, the dentate nucleus, the SNr and ALM to the lateral superior colliculus with the ALM thalamo-cortical loop on the basis of the overlaps in the anterograde fluorescence from a source area with ALM-projecting thalamus. In each case, quantifications of the anterograde fluorescence from a source area were always made in brains that contained co-injections of
tracers in the ALM to label ALM-projecting thalamus. Injections of retrograde (Ctb) and anterograde (BDA) tracers in the ALM produced similar patterns of labelling in the thalamus (Extended Data Fig. 8). We therefore used both types of tracers to label ALM-projecting thalamus. Quantifications of fluorescence were performed on images after alignment to the CCF anatomical template (Extended Data Fig. 7b). We found consistent labelling patterns in the thalamus across different injection cases (Extended Data Fig. 7c). Alignment to the CCF enabled us to combine quantifications of fluorescence overlaps across different injection cases (Fig. 4a–c, Extended Data Fig. 7d–f).

To quantify fluorescence overlaps with ALM-projecting thalamus, we first limited the analysis to a subregion of the coronal section that contained the thalamus ipsilateral to the ALM injection (shown in Extended Data Fig. 7b at different rostro-caudal levels). We then thresholded the fluorescence intensities independently for each channel at approximately the 89th percentile (85th–94th across different cases). The labelled area was defined as all the pixels that exceeded this threshold (Extended Data Fig. 7e). Finally, the overlap in fluorescence was computed as the number of pixels co-labelled by ALM injections and a projection (for example, the fastigial nucleus) divided by the total number of pixels labelled by that projection (‘area fraction’ overlap, Extended Data Fig. 7f). This procedure was carried out separately for each coronal section, which constituted one data point in Fig. 4c and Extended Data Figs. 7e, f, 8b.

Because we always performed triple injections (a retrograde or anterograde tracer in ALM, and anterograde tracers in two source areas—that is, the fastigial and dentate nucleus, or the fastigial nucleus and SNr, or the fastigial nucleus and superior colliculus, or other combinations), quantifications of fluorescence overlaps were further validated by visualizing all three channels in individual injection cases (Extended Data Fig. 8).

Statistics. The sample sizes are similar to sample sizes used in the field: for behaviour, three mice or more per condition; for electrophysiology, more than 100 units per brain region. No statistical methods were used to determine sample size. In one of the fastigial photoactivation mice, the injection of ChR2 virus missed the CN and its data were excluded. All key results were replicated in multiple mice. Mice were randomly preallocated into experimental groups. Unless stated otherwise, the investigators were not blinded to allocation during experiments and outcome assessment. Trial types were randomly determined by a computer program. During spike sorting, experimenters cannot tell the trial type, so were blind to the conditions. Statistical comparisons using t-tests and bootstrap are described in detail in the previous sections.

Code availability. Software used for data analysis is available from the corresponding author upon reasonable request.

Data availability
Raw and processed data are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Histology and behavioural data.

a, Coronal sections showing CN lesion sites for all mice. Fastigial lesion, n = 4; dentate lesion, n = 4. b, Execution of the licking movement is not affected by fastigial lesions. Individual licks are shown as dots (blue, lick right; red, lick left). Licks are aligned to the onset of the ‘go’ cue (vertical line).

c, Average lick rate within a lick bout and the duration of lick bouts are not affected by fastigial lesion. A lick bout is defined as a sequence of licks with an inter-lick interval of less than 500 ms. Data from the fastigial-lesion mice only (n = 4). Data are grouped by licking direction relative to the lesioned hemisphere: contralesional licking (blue) or ipsilesional licking (red).

d, Coronal sections showing ChR2–eYFP expression in the fastigial nucleus and the dentate nucleus. Nine mice were used for various fastigial ChR2 photoactivation experiments, and ten mice were used for dentate ChR2 photoactivation experiments. In one of the fastigial photoactivation mice, the injection of ChR2 virus missed the CN (not shown) and its data were excluded. Two of the dentate photoactivation mice were subsequently used for lesion experiments (not shown). See Supplementary Table 1 for the list of mice and manipulated hemispheres in individual experiments. e, CN photoactivation during specific behavioural periods. Top, the experiment timeline. Photostimulation was for the entire task period (1.3 s). Bottom, performance in the behavioural trials. Left, fastigial photoactivation (n = 6); right, dentate photoactivation (n = 8). For fastigial photoactivation, average behavioural performance for control and photoactivation trials (S, sample period photoactivation; D, delay period; R, response period) is also shown. Grey lines represent individual mice. Chance is 50%. For behavioural performance grouped by trial type, lick-left and lick-right trials are grouped by instructed licking direction relative to the manipulated hemisphere. Blue, contralateral; red, ipsilateral. Both hemispheres were tested. See Supplementary Table 1 for the list of mice and manipulated hemispheres. Thick lines represent the mean; thin lines represent individual mice (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001, one-sided test, bootstrap (Methods). f, Proportion of early-lick and no-lick trials with and without CN photoactivation during the delay period. Control trials are shown in grey and photoactivation trials in cyan. Lines represent individual mice. FN, fastigial photoactivation (n = 6); DN, dentate photoactivation (n = 8).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Characterization of CN photoinhibition and behavioural data. a, Strategy for silencing the CN. ChR2 is expressed in cerebellar Purkinje cells, and the photostimulation of Purkinje cells inhibits CN neurons (photoinhibition). Top left, an image of the cerebellar cortex showing the expression of ChR2–eYFP in Purkinje cells in a L7-cre × Ai32 mouse. Top right, an example silicon-probe recording in a L7-cre × Ai32 mouse. The coronal section shows ChR2–eYFP expression in the cerebellar cortex and DiI-labelled electrode tracks. Bottom, electrode tracks within the fastigial nucleus (dashed contour). Photostimulation of Purkinje cells was carried out by directing a blue laser (0.5–4.5 mW average power, beam diameter 400 μm at 4σ) to the brain surface through a cranial window. c, Example voltage traces from a recording of the fastigial nucleus during photoinhibition. Multi-unit activity in the fastigial nucleus was silenced. Multiple traces show repeats of photostimulation. d, Photoinhibition reduced activity specifically in the CN. Average spike rates before (500 ms) versus during photoinhibition (1.2 s) are shown, for neurons from the fastigial nucleus (left, n = 26) and from the surrounding cerebellar cortex (right, n = 34). Photostimulation of the cerebellar cortex (lambda posterior 3 mm, lateral 2 mm) silenced fastigial activity whereas photostimulation of the inferior colliculus (posterior 1 mm, lateral 2 mm) did not. Solid dots indicate neurons with a significant change in spike rate (P < 0.01, two-tailed t-test). e, Dose-dependent effect of CN photoinhibition on posture and movement. Left, video frames showing an unrestrained mouse undergoing CN photoinhibition. During low-intensity photoinhibition (1.5 mW, the intensity typically used in the delayed response task), no consistent movement was detected. High-intensity photoinhibition (20 mW) of both the fastigial nucleus and the dentate nucleus produced dystonia-like movements and posture changes, such as loss of balance and extensions of the contralateral limbs (arrows). Right, dystonia-like movements observed upon photoinhibition were given scores on a scale of 0–4 (Methods): 0, no motor abnormalities; 1, slightly slowed or abnormal motor behaviour, no dystonia; 2, mild impairment, mild and transient dystonic postures, weak tremor; 3, moderate impairment, dystonic postures, cannot balance the body, major tremor; 4, severe impairment, sustained dystonic postures and limited movements. The scoring was performed blind to the experimental conditions. Data are mean ± s.e.m. across trials. n = 2 mice, 10 trials per condition. f, Dose-dependent effect of CN photoinhibition on jaw movement and eyeblink. Top, video frames showing a head-fixed mouse undergoing CN photoinhibition. Bottom left, eyeblink responses during CN photoinhibition. No consistent eyeblink was evoked. Bottom right, jaw movements during CN photoinhibition. Only high-intensity photoinhibition (20 mW) of the dentate nucleus produced jaw movements. Data are mean ± s.e.m. across trials. n = 4 mice, 10 trials per condition for eyeblink, 20 trials per condition for jaw movement. g, Behavioural performance in the delayed response task with and without fastigial photoinhibition for the entire task periods (n = 3). Left, average behavioural performance for control and photoinhibition trials (S, sample period photoinhibition; D, delay period; R, response period). Grey lines show individual mice. Chance is 50%. Right, behavioural performance for each trial type. Lick-left and lick-right trials are grouped by instructed licking direction relative to the manipulated hemisphere. Blue, contralateral; red, ipsilateral. Both hemispheres were tested. See Supplementary Table 1 for the list of mice and manipulated hemispheres. Thick lines represent the mean; thin lines represent individual mice. *P < 0.05, **P < 0.01, ***P < 0.001, one-sided test, bootstrap (Methods). h, Behavioural performance in trials with photoinhibition during the early sample, late sample, early delay, and late delay periods (n = 7 mice). *P = 0.04, ***P = 0.0002, one-sided test, bootstrap. i, Proportion of early-lick and no-lick trials with fastigial photoinhibition during the delay period. Control trials (grey) and photoinhibition trials (cyan). Lines, individual mice (n = 7).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Silicon-probe recordings in the CN. a, Dil-labelled recording tracks in coronal sections stained by DAPI. Borders of the CN are visible in DAPI staining. Electrode location was estimated based on Dil labelling and manipulator depth. The lamination of activity patterns across the electrodes corresponded well to the anatomical structures: that is, high activity levels in the CN and the cerebellar cortex, low activity levels in the white matter. Single-unit locations were estimated on the basis of electrode location. Top, a coronal section showing a recording in the fastigial nucleus. Bottom, the fastigial nucleus (yellow dashed line), estimated electrode location, and the locations of single units (units 1–6) from an example recording. b, Left, voltage trace from the recording site of unit 1 during a single behavioural trial. Dashed lines indicate behavioural periods. Right, waveforms of unit 1 after spike sorting. c, Peristimulus time histogram of all the units from the example fastigial recording. The dashed box indicates that units 1–5 were within the fastigial nucleus, and unit 6 was outside of the fastigial nucleus. Blue, lick-right trials; red, lick-left trials. d, Same as a and c, but for an example recording in the interposed nucleus. e, Same as a and c, but for an example recording in the dentate nucleus.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | ALM and CN activity is related to motor planning and movement. a, Criteria used to identify neuronal activity modulated by licking. For each lick, spike counts were calculated in two adjacent 50-ms time windows, starting 20 ms from the detection time of the lick. Across all licks and all trials, neurons with significant difference in spike count between the two time windows were deemed to be modulated by licking ($P < 0.01$, two-tailed $t$-test). Licking modulation is calculated for each neuron: mean difference in spike rate between the two time windows. b, Example neurons are significantly modulated by licking. Peristimulus time histogram from the correct lick-right and lick-left trials during the response period. Spike times are aligned to the first lick. Trials are grouped by licking direction relative to the recorded hemisphere: contralateral (blue) or ipsilateral (red). Averaging window, 10 ms. c, Fraction of neurons that are significantly modulated by licking ($P < 0.01$, two-tailed $t$-test) from each brain region. FN, fastigial nucleus; IP, interposed nucleus; DN, dentate nucleus. Error bars indicate s.e.m. across mice, bootstrap (Methods); dots represent individual mice (ALM, $n = 10$; FN, $n = 15$; IP, $n = 9$; DN, $n = 11$). d, There is no correlation between whether a neuron was modulated by licking and whether it exhibited preparatory activity. Top, ALM; bottom, the cerebellar nuclei. Selectivity during the delay period is the difference in spike rate between the lick-right and lick-left trials. Solid dots indicate neurons that are significantly modulated by licking ($P < 0.01$, two-tailed $t$-test). e, Preparatory activity in the CN. Left, Peristimulus time histograms for correct and error trials are shown for two example CN neurons. Trial types are based on sensory instruction (blue, lick right; red, lick left). The same trial-type preference in correct and error trials indicates selectivity for object location. Opposite trial-type preference indicates selectivity for upcoming movement directions. A negative ratio of trial-type selectivity between error and correct trials means a neuron switches trial-type preference to predict upcoming movement directions on error trials. Right, selectivity ratios for CN neurons. Neurons with significant delay-period selectivity and tested for >3 error trials are shown ($n = 73$). f, Population selectivity for each cerebellar nucleus (mean ± s.e.m. across neurons, bootstrap; fastigial, $n = 87$; interposed, $n = 50$; dentate, $n = 60$). Selectivity is the difference in spike rate between the preferred and the non-preferred trial type (Methods). Dashed lines separate sample, delay and response periods. g, Proportion of contra-preferring versus ipsi-preferring neurons, based on spike counts during the first 500 ms (left) and the last 500 ms (right) of the delay period. Error bars indicate s.e.m. across mice. Dots represent individual mice (ALM, $n = 10$; FN, $n = 15$; IP, $n = 9$; DN, $n = 11$). ***$P = 0.0001$, one-sided test, bootstrap.
Extended Data Fig. 5 | ALM drives CN preparatory activity and vice versa. **a**, Top, schematic showing CN recording during bilateral ALM photoinhibition. Laser power, 1.5 mW per location (Methods). Bottom, Peristimulus time histograms of four example CN neurons with and without ALM photoinhibition. The cyan bar indicates the photoinhibition period. Blue, lick-right trials; red, lick-left trials. **b**, Left, average fastigial selectivity in control and photoinhibition trials (mean ± s.e.m. across neurons, bootstrap). Only selective neurons tested for >3 trials in all conditions are included (n = 54 neurons, 8 mice). Orange dashed line denotes the mean from control trials. Right, relationship between delay-period selectivity of individual fastigial neurons and changes in firing rate due to ALM photoinhibition. Filled circles indicate neurons that are significantly modulated by ALM photoinhibition (P < 0.01, two-tailed t-test). **c**, Same as **b**, but for the interposed nucleus (n = 44 neurons, 5 mice). **d**, Same as **b**, but for the dentate nucleus (n = 59 neurons, 6 mice). **e**, Same as **a**, but for ALM recording during contralateral CN photoinhibition. Laser power, 1.5–4.5 mW.
Extended Data Fig. 6 | CN preparatory activity is driven by both hemispheres of the ALM. a, CN recording during contralateral or ipsilateral ALM photoinhibition. b, Performance in the delayed response task during unilateral and bilateral ALM photoinhibition. c, CN population selectivity from control and photoinhibition trials (mean ± s.e.m. across neurons, bootstrap). Only selective neurons tested for >3 trials in all conditions are included (\( n = 157 \)). The orange dashed line indicates the mean from control trials. The cyan bar denotes the photoinhibition period. CN selectivity was reduced by photoinhibiting either side of the ALM. d, Peristimulus time histograms of example CN neurons during ipsilateral or contralateral ALM photoinhibition. The effect was heterogeneous across individual neurons. Some neurons were affected by ipsilateral ALM photoinhibition (row 1–3), other neurons were affected by contralateral ALM photoinhibition (row 4), others were affected by photoinhibition of either side (row 5, 6). e, Peristimulus time histograms of example CN neurons during bilateral ALM or M1 photoinhibition. ALM: bregma anterior 2–3 mm, lateral 1–2 mm; M1 anterior 0–1 mm, lateral 1–2 mm.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Alignment of anatomical data and comparing fastigial and dentate projections to ALM-projecting thalamus.

a, Top, an example coronal section before and after alignment to the corresponding section of the anatomical template in the Allen Mouse Common Coordinate Framework (CCF). Fluorescence is anterograde labelling (BDA) from an ALM injection, labelling the ALM-projecting thalamus (Methods). Yellow dots indicate control points for b-spline transformation. The dashed line denotes a region of interest containing the thalamus ipsilateral to the ALM injection. Bottom, overlay of the aligned image (orange) and the anatomical template (green). ALM axons in the thalamus obey structural boundaries in the anatomical template. Critically, the alignment procedure did not use any structures inside the thalamus as landmarks.

b, Coronal sections showing an example fastigial injection case with anterograde labelling in the thalamus. The sections are aligned to and superimposed on the anatomical template. The yellow outlines show labelled areas after thresholding (Methods).

c, Coronal sections showing three different fastigial injection cases. Different injections show similar patterns of anterograde labelling in the thalamus.

d, Left, outlines of the labelled areas from all fastigial injection cases (n = 6). Borders of the thalamic nuclei (VM and VAL) are based on annotations in the Allen Reference Brain. Right, outlines of the labelled areas from all fastigial (n = 6), dentate (n = 4) and ALM (n = 8) injection cases. There was a greater overlap between fastigial and ALM labelling.

e, Total areas labelled (number of pixels) in the thalamus by fastigial, dentate and ALM injections. Dots indicate individual coronal sections; lines represent individual injections.

f, Overlaps between fastigial or dentate projections and the ALM-projecting thalamus. The co-labelled area is normalized to the total thalamic area labelled by fastigial or dentate projections (area fraction overlap). Dots indicate individual coronal sections; lines represent individual injections.
Extended Data Fig. 8 | The ALM-projecting thalamus receives converging inputs from the fastigial nucleus, basal ganglia SNr and the superior colliculus. a, Example triple injections showing co-labelling of fastigial and dentate projections (left), fastigial and SNr projections (middle), and fastigial and superior colliculus projections (right) in the thalamus. Injections were repeated several times in different mice with similar results (ALM–fastigial–dentate, n = 4; ALM–fastigial–SNr, n = 3; ALM–fastigial–superior colliculus, n = 2). b, Overlaps between ALM-projecting thalamus and projections of different areas (Methods). Fastigial, SNr and superior colliculus labelling show a comparable amount of overlap with the ALM-projecting thalamus. Dentate labelling shows less overlap. The thalamic area co-labelled by ALM injection and a projection (fastigial, dentate, SNr or superior colliculus) is normalized to the total thalamic area labelled by that projection (area fraction). Dots indicate individual coronal sections; lines represent individual injections. c, A confocal image showing vGlut2 staining in the ALM-projecting thalamus. Anterograde tracer injections in the ALM (blue), the fastigial nucleus (green) and the SNr (red). Fastigial axons form glutamatergic synapses (vGlut2 positive) within the ALM-projecting thalamus. vGlut2 staining was performed in one ALM–fastigial–SNr injection case.
Extended Data Fig. 9 | CN ChR2 photoactivation drives rapid change in ALM activity. a, The change in firing rate of ALM neurons during fastigial or dentate ChR2 photoactivation. The change in firing rate is the difference in spike rate between control and photoactivation trials. For neurons that were suppressed by CN photoactivation, the firing rate differences are multiplied by $-1$ so that the firing rate changes are always positive for latency quantifications. Data are mean ± s.e.m. across neurons. Only neurons that were significantly modulated by CN photoactivation are included ($P < 0.01$, two-tailed t-test; fastigial photoactivation, $n = 227$; dentate photoactivation, $n = 163$). Top, Time course for the change in ALM firing rate. The cyan bar denotes the CN photoactivation period (500 ms). Bottom, The onset of the change in firing rate of ALM neurons (arrows). The change of firing rate is quantified in 10-ms time bins in 1-ms steps. The onset time is the first time bin in which the change in population firing rate significantly deviated from zero ($P < 0.01$, two-tailed t-test). We repeated the onset time estimation 10,000 times. In each round, we resampled with replacement from the neurons in the dataset and re-estimated the onset time. The arrows indicate the mean estimated onset time. b, Relationship between selectivity of individual ALM neurons and changes in firing rate due to CN photoactivation. Filled circles denote ALM neurons that were significantly modulated by CN photoactivation ($P < 0.01$, two-tailed t-test). The change in firing rate is the difference in the spike rate between control and photoactivation trials during photostimulation (top) or the last 500 ms of the delay period (bottom). The selectivity is the difference in spike rate between lick-right and lick-left trials during the delay period. c, Selectivity of the ALM population from control and photoactivation trials (mean ± s.e.m. across neurons, bootstrap; selective neurons tested for >3 trials in all conditions). The orange dashed line indicates the mean from control trials. Top, fastigial photoactivation ($n = 328$); bottom, dentate photoactivation ($n = 377$). The cyan bar denotes the photoactivation period.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Fastigial photoactivation abolishes the relationship between ALM activity and upcoming movements. a, ALM recording during bilateral ALM photoinhibition (top) or contralateral fastigial photoactivation (bottom). b, Performance in control (grey) and photostimulation (cyan) trials. Lick-left and lick-right trials are pooled. Chance is 50% (dashed line). Lines represent individual mice (ALM photoinhibition, n = 7; fastigial photoactivation, n = 6). ***P < 0.001, one-sided test, bootstrap (Methods). c, Left, schematic of ALM activity projected onto the coding direction (cd). Dashed lines indicate control trials, ALM activity trajectories converge to discrete endpoints at the end of the delay period that predict upcoming movement directions (lick right, blue; lick left, red). Solid black lines indicate bilateral ALM photoinhibition trials; the distance of the perturbed trajectories to the endpoints predicts future movement directions even though the behavioural performance is close to that which would be obtained by chance. Right, experimental data. Probability of the mouse licking right as a function of trajectory distance to the endpoints along the cd projection. The s.e.m. is obtained by bootstrapping the trials in each bin. d, Same as c, but for fastigial photoactivation trials (n = 337). ALM activity no longer predicts future movement directions after a fastigial perturbation. e, 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 the ALM (Methods). Left, unilateral ALM photoinhibition during the late delay period. Right, behavioural performance for each trial type in control and photoinhibition trials. Left ALM photoinhibition biased upcoming licking to the left, resulting in lower performance in the lick-right trials. The opposite pattern of bias was induced by right ALM photoinhibition. Thick lines represent the mean; thin lines represent individual mice (n = 4). ***P = 0.0002, one-sided test, bootstrap (Methods). f, Same as e, but for unilateral ALM photoinhibition after fastigial perturbation. Note the same pattern of behavioural bias as e. *P = 0.04, ***P = 0.008, one-sided test, bootstrap. g, Left, after fastigial perturbation, ALM activity was bilaterally silenced during movement initiation. Right, fraction of trials in which mice did not lick after the ‘go’ cue. FN, fastigial perturbation only; ALM, bilateral ALM photoinhibition; FN + ALM, fastigial perturbation followed by bilateral ALM photoinhibition. Thick lines represent the mean; thin lines represent individual mice (n = 3). **P = 0.002, ***P = 0.0002, one-sided test, bootstrap.
**Reporting Summary**

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- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- [ ] Clearly defined error bars
  - *State explicitly what error bars represent (e.g. SD, SE, CI)*

*Our web collection on statistics for biologists may be useful.*

**Software and code**

**Policy information about availability of computer code**

**Data collection**
- Behavioral data were acquired using commercial hardware (Bpod, Sanworks) and software (https://github.com/sanworks/Bpod_Gen2).
- Electrophysiology data were acquired using Open-Source RHD2000 Interface Software from Intan Technology (version 1.5.2, http://intantech.com/downloads.html#software).
- Anatomy data were acquired using a commercial confocal microscope (LSM 700, Carl Zeiss) operated with Zeiss Zen Software (v2.2).

**Data analysis**
- Behavior and electrophysiology analyses were performed in Matlab using custom scripts and published analytical methods.
- Anatomy images were processed in Fiji ImageJ software (v.1.46).
- Anatomy data were aligned to the Allen Mouse Common Coordinate Framework using custom Matlab scripts and the B-spline Grid, Image and Point based Registration package available on the Matlab FileExchange (https://www.mathworks.com/matlabcentral/fileexchange/20057-b-spline-grid--image-and-point-based-registration).
- Custom analysis scripts and data will be deposited on CRCNS.org.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

The sample sizes are similar to sample sizes used in the field: for behavior, 3 mice or more per condition; for electrophysiology, more than 100 units per brain region.

Data exclusions

In 1 of the fastigial-stimulation mice, the injection of ChR2 virus missed CN and its data were excluded.

Replication

All key results were replicated in multiple mice.

Randomization

Mice were randomly pre-allocated into experimental groups.

Blinding

Unless stated otherwise in specific experiments, the investigators were not blinded to allocation during experiments and outcome assessment. Trial types were randomly determined by a computer program. During spike sorting, experimenters cannot tell the trial type, so experimenters were blind to conditions.

Reporting for specific materials, systems and methods

Materials & experimental systems

- n/a Involved in the study
- ✔ Unique biological materials
- ✔ Antibodies
- ✔ Eukaryotic cell lines
- ✔ Palaeontology
- ✔ Animals and other organisms
- ✔ Human research participants

Methods

- n/a Involved in the study
- ✔ ChIP-seq
- ✔ Flow cytometry
- ✔ MRI-based neuroimaging

Antibodies

Antibodies used

Mouse anti-NeuN (Millipore, MAB337, lot 2654334)
Chicken anti-GFP (Aves, GFP-1020, lot GFP697986)
Rabbit anti-RFP (Rockland, 600-401-379, lot 36815)
Mouse anti-vGlut2 (Millipore, MAB5504, lot 2012363)

Validation

Mouse anti-NeuN: the immunofluorescent labeling has been validated with mouse brain slices. (http://www.merckmillipore.com/NL/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377, see also Figure 1 and Extended Data Figure 1 in this study).
Chicken anti-GFP: the immunofluorescent labeling has been validated with mouse brain sections. (http://www.aveslab.com/products/epitope-tag-and-gfp-antibodies/anti-gfp-green-fluorescent-protein-antibodies-2/, see also Extended Data Figure 8 in this study).
**Animals and other organisms**

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals                                                                 |                                                                 |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------|
| This study is based on data from 70 mice (age > P60, both male and female mice, Supplemental Table 1): |                                                                 |
| 13 C57B1/6 mice were used for cerebellar nuclei (CN) ChR2 photo-activation experiments. A subset of these mice (10 mice) were used for ALM recordings during photo-activation of the CN. |                                                                 |
| 8 C57B1/6 mice were used for CN lesion experiments.                                 |                                                                 |
| 7 L7-cre × Ai32 (Rosa26-LSL-Chr2-EYFP, JAX Stock#012569) mice were used for CN photo-inhibition experiments during behavior. A subset of these mice (4 mice) were used for ALM recordings during CN photo-inhibition. |                                                                 |
| 2 L7-cre × Ai32 mice were used to characterize CN photo-inhibition using electrophysiology. |                                                                 |
| 6 L7-cre × Ai32 mice were used to quantify general effects of CN photo-inhibition on movements. |                                                                 |
| 10 C57B1/6 mice were used for CN recording experiments.                              |                                                                 |
| 8 VGAT-ChR2-EYFP mice (Jackson laboratory, JAX Stock#014548) were used for CN recordings during ALM photo-inhibition. |                                                                 |
| 4 VGAT-ChR2-EYFP mice were used for behavioral experiments in which both ALM and CN activity were manipulated to test the causal role of ALM activity in behavior following a fastigial perturbation. |                                                                 |
| 12 C57B1/6 mice (not listed in Supplemental Table 1) were used for anatomical tracing. |                                                                 |

| Wild animals                                                                 | The study did not involve wild animals                             |
| Field-collected samples                                                     | The study did not involve samples collected from the field.       |