Short latency cerebellar modulation of the basal ganglia

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Basal ganglia and cerebellum are crucial for purposeful voluntary movement and perform related, but distinct and complementary, functions. Their dysfunction leads to diverse motor disorders ranging from Parkinson’s and Huntington’s disease to loss of balance and ataxia. Both structures are part of parallel multi-synaptic motor control loops that receive input from and send information back to the cortex. To optimally control movements that require exquisite timing precision, it is beneficial for these functionally related structures to communicate and coordinate their outputs in real time. Anatomical tracing studies in rodents and primates have revealed a direct disynaptic projection from the cerebellar nuclei to the basal ganglia via the intralaminar thalamic nuclei. This disynaptic pathway can, in principle, provide a short-latency conduit for fast communication between the cerebellum and basal ganglia, thereby enabling them to quickly coordinate their outputs. However, electrophysiological studies in anesthetized cats have only revealed slow- and long-latency responses in the basal ganglia following strong, repeated electrical stimulation of the cerebellum. To date there is no functional evidence for the presence of rapid communication between the cerebellum and the basal ganglia. Thus, whether cerebellum and basal ganglia can communicate rapidly, and indeed the utility of the disynaptic pathway as a short-latency conduit between the two structures, remains to be established.

In freely moving mice, we found that cerebellar stimulation effectively altered the activity of striatal neurons in about half of the neurons examined. Cerebellar modulation of striatal neurons had a short latency of \(-10\) ms and was mediated via the disynaptic cerebello-thalamo-striatal pathway. We explored whether this pathway is capable of altering corticostriatal plasticity. We found that high-frequency stimulation of the cortex alone predominantly depressed corticostriatal responses. However, concurrent activation of the cerebellum during high-frequency stimulation of the cortex altered the direction of the plasticity, resulting in the long-term potentiation of corticostriatal responses. We also found that, under pathological conditions, the disynaptic pathway allowed for the propagation of aberrant cerebellar activity to the basal ganglia. Thus, we found that, in an animal model of cerebellar-induced dystonia, as a consequence of aberrant cerebellar output, the activity of striatal neurons was driven to high-frequency burst firing. Acutely severing the link between the cerebellum and basal ganglia by silencing the appropriate thalamic nuclei resulted in rapid alleviation of the dystonic symptoms. Our data provide evidence in support of short-latency communication between the cerebellum and the basal ganglia and suggest that the pathway has a crucial role in motor function and dysfunction.

**RESULTS**

**Short-latency cerebellar modulation of the basal ganglia**

To examine whether a short-latency connection between the cerebellum and the basal ganglia exists, we monitored the single-unit activity of neurons in the basal ganglia in awake freely moving mice while electrically or optogenetically activating the contralateral cerebellar dentate nucleus (Figs. 1 and 2). Recordings were obtained from the dorsolateral striatum, the input nucleus of the basal ganglia, which, on the basis of anatomical tracings, is the primary target of the disynaptic cerebellar projections from the dentate nuclei. Relatively weak electrical or optogenetic stimulation of the cerebellum (mean stimulation threshold, electrical = 45 ± 2 µA, optogenetic = 2.3 ± 0.5 mW; Supplementary Figs. 1 and 2) rapidly altered the activity of striatal neurons in about half the cells that we examined (electrical, 78 of 154 neurons (n), 14 of 14 mice (N); optogenetic, 17 of 33 neurons, 4 of 4 mice) by increasing their firing rate, decreasing it or a combination thereof (Figs. 1 and 2). In all cases, the extent to which the firing rate increased or decreased was correlated with the strength of cerebellar stimulation (Fig. 1b,c); on average, increasing the stimulation intensity by \(\pm 20\) µA more than tripled the strength of excitatory responses (Supplementary Fig. 2).

The striatum contains at least four major cell types: medium spiny neurons (MSNs), fast spiking GABAergic interneurons (FSIs),...
persistent low threshold interneurons and cholinergic interneurons. It has been suggested that a neuron’s average firing rate and spike waveform in vivo can be used to identify it as either a MSN or FSI. MSNs typically have low firing rates and broad waveforms, whereas FSIs on average fire at higher rates and have briefer waveforms. Cholinergic interneurons are thought to have features that are somewhat in between the two. On the basis of the distribution of the spike waveform and firing rate, we determined that the responsive cells were not restricted to FSIs or MSNs and were distributed throughout the data set (Fig. 3).

To examine how fast striatal neurons respond to cerebellar stimulation, we measured response latencies in cells which were excited by cerebellar stimulation. Responses occurred rapidly after cerebellar stimulation, with latencies ranging from 3–28 ms and excitatory responses having a mean latency of 10.3 ± 5.8 ms and a median latency of 9.0 ms (n = 73; Fig. 3g). Cells recorded simultaneously on the same electrode or adjacent electrodes could have different response latencies with no correlation between presumed cell type (tentatively assigned on the basis of their spike waveforms and baseline firing rate) and response latency.

Cerebellar inputs to the basal ganglia are mediated by the intralaminar nuclei of the thalamus

The short latency of cerebellar-induced striatal responses is compatible with them being mediated by the direct cerebello-basal ganglia disynaptic pathway routed through the intralaminar thalamic nuclei.

We employed two parallel approaches to test this possibility. With the first approach, we optogenetically activated the cerebellar projection axons in the intralaminar nuclei and examined the resulting responses in the activity of striatal neurons (Fig. 3d). Selective activation of cerebellar axons in the intralaminar thalamic nuclei was as effective as direct stimulation of the dentate nuclei in altering the activity of striatal neurons and produced comparable response types and latencies in the majority of the cells examined (14 of 20 cells examined, N = 3; Fig. 3d,e). With the second approach, we examined the consequences of inactivating the intralaminar thalamic nuclei (contralateral to the stimulation site) on...
cerebellar-induced striatal responses. Localized inactivation of thalamic nuclei by acute injection of QX314 or tetrodotoxin (TTX), blockers of voltage-gated sodium channels that prevent action potential generation and propagation, reversibly abolished cerebellar-induced responses in striatal neurons (7 of 8 attempts, 24 of 27 cells, N = 4; Fig. 4a and Supplementary Fig. 3). These data support the hypothesis that thalamic neurons are required to convey cerebellar information to the basal ganglia. However, it is also possible that the sodium channel blockers also blocked action potential propagation in axons of passage that happen to course close to the injection site. To address this possibility, we optogenetically silenced intralaminar thalamic neurons and found that this was also effective at blocking striatal responses to optogenetic stimulation of cerebellar axons in the CL (n = 20, N = 3). (f) Firing rate and waveform characteristics of every dorsolateral striatal neuron that showed a response (red) or was unaffected (black) by electrical or optogenetic stimulation of the cerebellum. (g) Example responses to optogenetic stimulation of cerebellar projection axons in the CL and surrounding intralaminar thalamic nuclei. (e) Percentage of cells responding with each response type to optogenetic stimulation of cerebellar axons in the CL (n = 20, N = 3). (f) Firing rate and waveform characteristics of every dorsolateral striatal neuron that showed a response (red) or was unaffected (black) by electrical or optogenetic stimulation of the cerebellum. (g) Excitatory response latencies of striatal neurons following cerebellar activation using electrical DN stimulation, optogenetic DN stimulation or optogenetic stimulation of cerebellar axons in the thalamus.

It remains plausible, although perhaps somewhat unlikely, that the cerebellar-induced striatal responses were mediated by a thalamo-cortical pathway. In this case, one could imagine that the cortex was excited by the thalamus as it relayed cerebellar information18,19. This increased cerebellar-induced cortical activity could then subsequently activate striatal neurons. To examine this possibility, we inactivated the cortex by acutely injecting TTX or QX314 through a cannula positioned in the motor cortex. Cortical inputs provide a prominent input to the basal ganglia20 and, as expected, inactivation of the cortex substantially (~50%) reduced the spontaneous firing rate of the striatal neurons (Fig. 4c). Nonetheless, after cortical inactivation, cerebellar stimulations remained effective at producing short-latency responses in all of the striatal neurons that we examined (n = 5 cells, N = 2; Fig. 4c). In three additional animals, the motor cortices were surgically removed bilaterally (Supplementary Fig. 5), and the response of neurons in the dorsolateral striatum to optogenetic stimulation of the cerebellum was examined 3 weeks later. Even with motor cortices removed, striatal neurons responded to cerebellar stimulation, with excitatory responses having an average latency of 8.4 ± 1.2 ms (n = 5 single-unit and 24 multi-unit recordings, N = 3; Fig. 4d). Collectively, these data suggest that the short-latency responses seen in dorsolateral striatum with cerebellar stimulations are most likely mediated by a direct disynaptic pathway independently of the cortex.
Cerebellar modulation of corticostriatal plasticity

In addition to allowing real-time communication between cerebellum and basal ganglia, the short-latency conduit might also be involved in motor learning. It is well known that both cerebellum and the basal ganglia are required for motor learning, and the disynaptic cerebello-basal ganglia pathway may provide a mechanism by which the basal ganglia combine cerebellar information to optimize motor learning. In this context, the reduced performance of rodents when the disynaptic connection between the two structures is severed may be, at least partially, a result of suboptimal learning. This hypothesis would also account for the fact that many motor learning procedures seem to critically require both the cerebellum and the basal ganglia. Given that the fidelity of synaptic communication between the motor cortex and the basal ganglia is thought to have a prominent role in motor learning, we considered the possibility that the properties and extent of cortico-striatal plasticity might be dependent, to some degree, on cerebellar input. It is known that, although selective high-frequency stimulation of the cortico-striatal pathway typically results in its long-term depression (LTD), the pathway can also undergo long-term potentiation (LTP), for example, if the target striatal neuron is concurrently depolarized. We therefore explored whether cerebellar activity could substitute for depolarization of the target striatal neuron to regulate the direction of cortico-striatal plasticity.

We found, in awake freely moving mice, that activation of the cortex yielded smaller responses following high-frequency electrical stimulation of the cortico-striatal pathway in the majority of the cells (22 of 27 cells, N = 7; Fig. 6a). Similar to that seen with striatal synaptic LTD in vitro, this weakening of the cortico-striatal response required CB1 receptors and was sensitive to intraperitoneal injection of the CB1 receptor antagonist AM-251 (n = 23, N = 3; Fig. 6b,c). We then examined whether co-activation of the cerebellum altered the extent or direction of the cortico-striatal plasticity seen following high-frequency stimulation of the cortex. We found that, in cells that were responsive to both cortical and cerebellar stimulation, when the same high frequency cortico-striatal stimulation was combined with...
Inhibition dominates in some striatal cells and inhibition dominated in others. The last stimulus pulse at the end of each train was monitored to determine whether excitation persisted (examples in a and b), or inhibition dominated (50 Hz example shown in c). (d) Both with 25- and 50-Hz trains, whether excitation or inhibition dominated during the train was correlated with the baseline firing rate of the striatal neuron; excitation was dominant in cells with higher baseline firing rates cells (**P < 0.01, two-tailed Mann-Whitney U test). Data are presented as mean ± s.e.m.

Figure 5 Striatal neurons can follow high-frequency cerebellar stimulation. To examine whether the striatum can follow high-frequency cerebellar activity, we electrically stimulated the dentate nucleus with 25- and 50-Hz trains. All of the neurons examined (n = 32, N = 5) could follow the high-frequency trains. (a–c) Example responses for different cells to a single pulse, 25-Hz train and 50-Hz train at the same stimulation intensity. With high-frequency stimulation, excitatory responses dominated in some striatal cells and inhibition dominated in others. The last stimulus pulse at the end of each train was monitored to determine whether excitation persisted (examples in a and b), or inhibition dominated (50 Hz example shown in c). (d) Both with 25- and 50-Hz trains, whether excitation or inhibition dominated during the train was correlated with the baseline firing rate of the striatal neuron; excitation was dominant in cells with higher baseline firing rates cells (**P < 0.01, two-tailed Mann-Whitney U test). Data are presented as mean ± s.e.m.

Dynamic cerebellar-basal ganglia interactions in a mouse model of rapid-onset dystonia Parkinsonism

The disynaptic cerebello-basal ganglia pathway has also been suggested to have a prominent, yet adverse, role under pathological conditions. For example, in an animal model of rapid onset dystonia-Parkinsonism (RDP), which is caused by loss-of-function mutations in the sodium pump30, the disynaptic pathway has been speculated to be the conduit through which aberrant cerebellar activity alters the basal ganglia to cause dystonia, as severing the communication by lesioning the centrolateral nucleus of the thalamus (CL, one of the intralaminar nuclei in rodents that hosts the bulk of the disynaptic connections19) prevents cerebellar induction of dystonia31. Given the strong ability of the cerebellum in modulating basal ganglia activity described here, we wondered whether cerebellar-induced dystonia is a consequence of rapid and continuous dynamic interactions between the two structures. To directly test this hypothesis, we used a previously established mouse model of RDP31 to examine the activity of striatal neurons when dystonia was induced by selective partial block of cerebellar sodium pumps. We found that cerebellar-induced dystonic postures, monitored visually and by field electromyogram (fEMG) recordings, were temporally correlated with abnormal neuronal activity in the dorsolateral striatum (N = 7; Fig. 8a). During these dystonic postures, the activity of striatal neurons was transformed to high-frequency burst firing (n = 139 cells under control and 42 cells under dystonic conditions, N = 12; Fig. 8b–e), mimicking the bursting activity seen in the basal ganglia of dystonic patients32. Moreover, consistent with the premise that the striatal neurons were driven by a common aberrant synaptic drive, there was a substantial increase in cross correlation of the activity of neighboring neurons during the cerebellar-induced dystonic postures (Fig. 8f,g), with no obvious association between the striatal neurons’ spike waveform characteristics and the severity of dystonia-induced burst firing. Lastly, we found that severing the link between the cerebellum and the basal ganglia by either electrically lesioning or optogenetically silencing the intralaminar thalamic neurons bilaterally by primarily targeting CL alleviated cerebellar-induced dystonia (N = 6 mice for CL lesions, 3 mice and 5 trials for optogenetic inactivation of CL; Fig. 8h and Supplementary Video 1). In the case of optogenetic silencing of the intralaminar thalamic neurons, dystonia abated within minutes if not seconds of laser activation and returned soon after termination of the light pulse.
Figure 6 Corticostratal plasticity in vivo. (a) Single-unit activity of dorsolateral striatal neurons was recorded in awake freely moving mice in response to activation of the motor cortex before and after high-frequency stimulation (HFS) of the cortex (100 Hz). Data are presented as the ratio of the number of spikes after high-frequency cortical stimulation (Aspikes post-stimulus) to that before HFS (Aspikes pre-stimulus) with the scatter of individual cells on the left. A ratio greater than 1 indicates LTP (light blue symbols) and less than 1 indicates LTD (dark blue symbols) (mean ± s.e.m.; LTD, n = 21; LTP, n = 5; N = 4; *P < 0.05, ****P < 0.0001, one-tailed, one-sample Wilcoxon signed ranks). (b) AM-251 was injected intraperitoneally 30 min before delivery of cortical HFS to test whether the plasticity induced by motor cortex HFS was cannabinoid dependent. AM-251–injected animals showed significantly more LTP than LTD (one-tailed, one-sample Wilcoxon signed ranks). (c) The magnitude of LTD in the animals injected with AM-251 was significantly less in the animals injected (one-tailed Mann-Whitney U test, *P < 0.05). In contrast, the magnitude of LTP in the animals injected with AM-251 was the same as that seen in the control animals (one-tailed Mann-Whitney U test, n.s. indicates not significant, P = 0.82).

DISCUSSION

The cerebellum and basal ganglia are intricately related to generating well-formed movements. However, there has been little functional evidence to suggest that they can directly exchange information at timescales relevant for fast movements that they sustain. We found that there is a prominent short-latency pathway from the cerebellum to the basal ganglia. Under physiological conditions, this pathway allowed for rapid communication between the cerebellum and the basal ganglia and permitted cerebellar modulation of corticostratal plasticity. Under pathological conditions, the pathway allowed for the transfer of aberrant cerebellar activity to the basal ganglia, causing movement disorders such as dyskinesia and dystonia.

Potential functional implications of the cerebellar inputs to the basal ganglia

Cerebellar activity correlates with movement kinematics and is necessary for the real-time adjustment and optimization of muscle activity. On the other hand, it is thought that the basal ganglia are primarily concerned with the selection of optimal motor commands. However, in the process of selecting the optimal motor command, it seems beneficial for the basal ganglia to have the most updated information about muscle kinematics. Thus, cerebellar inputs to the basal ganglia might be of value for correct action selection. Accordingly, the short-latency communication delineated here might simply be in place to enable the two structures to synchronize and coordinate their activities during movements that require a high degree of timing precision.

The cerebellum is also thought to act as a predictive computational device and the disynaptic connection may constitute a rapid pathway for communicating cerebellar predictions to the basal ganglia. In both cases, the need for a short-latency communication between the cerebellum and the basal ganglia is greatest when the cerebellum is expected to make a substantial contribution to motor coordination, for example, when movements are fast and complex. In support of this hypothesis, severing the disynaptic projection by selective lesion of the CL has been shown to reduce the performance of rats on a cerebellar intensive rotarod task by half without affecting their performance on stationary apparatus.

Cerebellar input to different striatal neurons classes

There are numerous classes of neurons in the striatum and these are thought to have distinct roles in information processing. On the basis of examination of the extracellular waveform and firing rates of the neurons from which we recorded, we did not find any evidence that there are discernable predictors of which cell types will or will not receive cerebellar input. However, these parameters only allow one to unambiguously distinguish between fast-spiking interneurons and MSNs. Thus, our data do not preclude the possibility that the cerebellum might have preferential input to subgroups in these populations. Anatomical tracings suggest that the disynaptic projection innervates the indirect pathway of the basal ganglia. This would suggest that the cerebellum at least communicates with D2 receptor–expressing MSNs. Whether it also has inputs to D1 receptor–expressing MSNs, or specific subtypes of interneurons, needs to be determined.

Corticostratal plasticity

We found that high-frequency stimulation of the cortex depressed corticostratal responses and, consistent with findings made in vitro,

\[
\Delta \text{Spikes}_{\text{post-stimulus}} \quad \Delta \text{Spikes}_{\text{pre-stimulus}}
\]

100-Hz HFS

\[
\text{CTL} \quad \text{AM251}
\]

\[
1 \quad 2 \quad 3 \quad 4 \quad 5
\]

\[
\text{LTD} \quad \text{LTP}
\]
that the plasticity seen was sensitive to block by the endocannabinoid antagonist AM251. In a small fraction of cells, however, the same high-frequency stimulation potentiated the corticostriatal responses. The LTP seen in these cells may be a consequence of push and pull interactions between LTD and LTP mechanisms. Alternatively, given the in vivo nature of our preparation (behaviorally active freely moving animals), it may represent the effect of neuromodulators on the circuit during specific behaviors.

We further found that simultaneous cortical and cerebellar high-frequency stimulations induced LTP. The precise mechanisms of LTP in corticostriatal inputs are unclear, although they are thought to involve postsynaptic NMDA receptors. It is plausible that cerebellar input depolarizes striatal cells and that this depolarization is sufficient to prime NMDA receptors for coincident input from the cortex. Alternatively, dopamine may be involved via intrastriatal release through cerebellar activation of interneurons or cerebellar activation of the substantia nigra.

Notably, our report is not the first hint of cerebellar involvement in corticostriatal plasticity. It has been shown that cerebellar LTD is not present in animals with contralateral cerebellar hemispherectomies. Given our findings, it may be that cerebellar-striatal potentiation balances corticostriatal depression; without cerebellar input, corticostriatal LTD saturates to a level at which further depression does not occur.

Cerebello-thalamo-striatal circuitry and dystonia
We found that, in an animal model of a cerebellar-induced dystonia, the neurons of the dorsolateral striatum exhibited abnormal high-frequency burst firing. Similar high-frequency burst firing has been noted in the basal ganglia of dystonic patients. Given these observations, it is possible that bursting activity in the basal ganglia may be the common substrate that causes dystonia in different dystonias, independent of whether the burst firing is caused by intrinsic defects in the basal ganglia or whether it is driven by aberrant input from other structures. On the basis of the data presented, it is also clear that acutely silencing the connection between the cerebellum and basal ganglia alleviates dystonia. Thus, in cases in which the cerebellum is known to cause dystonia, surgically disrupting (by lesioning or deep brain stimulation) the human thalamic nuclei that accommodate the disynaptic pathway may be a viable therapeutic approach. In fact, interventions involving the thalamus have been shown to produce immediate alleviation of dystonic symptoms in a number of patients.
**Figure 8** Cerebellar-induced dystonia is associated with dynamic interactions between the cerebellum and basal ganglia. (a) Simultaneous multi-unit (MUA) recording from dorsolateral striatum (top black trace) and fEMG (bottom green trace) in a mouse with cerebellar-induced dystonia. At the time indicated by the dashed red line, the animal suffered a severe dystonic posture and this was reflected both as an increase in the MUA and concurrently in the fEMG record. The regular spikes throughout the fEMG correspond with the heartbeat. (b) Single-unit activity of a striatal neuron in an awake freely moving mouse recorded under normal conditions (control, gray) and during cerebellar-induced dystonia (green). Scale bars represent 500 ms and 100 μV. (c) Average autocorrelation of striatal neuron activity before (gray) and after (green) cerebellar-induced dystonia revealed an increased tendency to burst firing during dystonia. The shaded areas denote s.e.m. (d) Average firing rate, mode of the instantaneous firing rate and the coefficient of variation of interspike intervals (ISI) in dorsolateral striatal neurons before (gray bars) and during (green bars) cerebellar-induced dystonia. With dystonia, the average firing rate of striatal neurons was not changed appreciably, whereas their burst firing was reflected in their high predominant (mode) firing rate and in their irregular activity (indicated by the increase in ISI coefficient of variation). Data are represented as mean ± s.e.m. (n = 139 control, 42 dystonia, ****p < 0.0001, two-tailed unpaired t test). (e) Rasters showing the spiking of representative striatal neurons recorded from control (black) and dystonic (green) mice. Each row represents 25 s and the same cell’s activity continues from one row to the next. The example rasters show the activity of the same two adjacent striatal neurons recorded by the same electrode before (gray) and after (green) cerebellar-induced dystonia. Each row represents 30 s and the cell pair’s activities continue from one row to the next. During dystonia there was a significant increase in the periods of time at which the two adjacent cells were active at the same time. (g) Average cross-correlation of activity of adjacent striatal cell pairs under normal conditions (black) and during cerebellar-induced dystonia (green). The shading represents s.e.m. (n = 21 pairs). (h) Left, dystonia scores in animals with cerebellar-induced dystonia before and after bilateral lesioning of the CL. Right, Nissl-stained section of tissue of a CL-lesioned animal and matching stereotaxic atlas. Lesion area is marked in red. Scale bar represents 1 mm. Data are presented as mean ± s.e.m. LV, lateral ventricle. *P < 0.05, Wilcoxon paired signed ranks. (i) Average dystonia scores before, during and after optogenetic silencing of the intralaminar thalamic nuclei in mice with cerebellar-induced dystonia. To silence neurons, archaerhodopsin was expressed in the intralaminar nuclei and CL was primarily targeted by the fiber optic. The photographs show cerebellar-induced dystonia in mouse (top) and its alleviation (bottom) when laser was turned on to silence CL. *P < 0.05, Wilcoxon paired signed ranks.

**Conclusions**

The robust and rapid cerebellar modulation of basal ganglia that we discovered provides a key insight as to how these two structures communicate in health and disease. Future scrutiny will no doubt unravel the nature of the information that is conveyed from the cerebellum to the basal ganglia and any topographic organization of this pathway. Given that both the cerebellum and the basal ganglia have also been implicated in non-motor tasks, it would be interesting to examine whether cerebellar modulation of basal ganglia activity also extends to its cognitive functions. It is interesting to note that a reverse cortex-independent pathway from the basal ganglia to the cerebellum has also been described anatomically. It would be important to determine the functional efficacy of this connection and to explore whether it provides a conduit for short-latency transfer of information in the opposite direction to that delineated here, thereby providing for bidirectional real-time communication between these two motor structures.

**METHODS**

Methods and any associated references are available in the online version of the paper.

*Note: Any Supplementary Information and Source Data files are available in the online version of the paper.*

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ONLINE METHODS

Experiments were conducted on 10–40-week-old C57/BL6 mice in accordance with guidelines set by Albert Einstein College of Medicine. Mice were housed on a 12-h:12-h reversed light/dark cycle. Mice were allowed to recover for at least four days after surgery and housed individually. When brain regions were stimulated or inhibited optogenetically, experiments began ≥2.5 weeks after surgery to allow for optimal opsin expression.

Single-unit microwire recordings and data analysis. All experiments were performed during the mouse’s dark cycle. Single-unit recordings were made unilaterally from dorsolateral striatum using custom-made eight wire drivable microarrays49. The microarray consisted of 100-µm Teflon insulated tungsten wires (50-µm core, AM Systems) assembled into two by four arrays (0.4 × 0.8 mm) and fixed into a custom-made screw drive (FineLine Prototyping). Arrays were implanted into the dorsolateral striatum (ML 2.5 mm, AP 0.55 mm, 1.2 mm from the surface of the brain).

Microwire arrays were advanced 75 or 150 µm per d as needed with a maximum total advancement of 1.5 mm. This range ensured that recordings were limited to the dorsal aspect of the striatum. The correct positioning of the recording and stimulation wires were ascertained histologically postmortem. To do so, at the end of the recording schedule a 60-µA, 30-s-long current was used to lesion the brain. Animals were then killed with a lethal dose of halothane and transcardially perfused sequentially with phosphate-buffered saline and 4% paraformaldehyde (wt/vol). Brains were extracted and fixed post-fixed in 4% paraformaldehyde for at least 24 h and then cryoprotected in 30% sucrose (wt/vol) for 1–3 d. Brains were sectioned at 50 µm and Nissl stained (Supplementary Fig. 1a,b).

Single unit signals were amplified 5,000× using a headstage (TuckerDavis Technologies) and a homemade amplifier (150 Hz to 10 kHz, RC bandpass filter) and digitized at 20 kHz with a National Instruments card (PCI-MIO-16XE) using custom-written software in Labview. Signals were wavelet filtered50 in Matlab (Mathworks) and sorted offline using principle component analysis (Offline Sorter, Plexon). Units with amplitudes <4× (signal σ) were excluded from the analysis. Waveforms were quantified in Matlab with valley and peak referring to the negative and the positive deflections of the waveforms corresponding with the rising phase and the downstream of the cell’s action potential.

Post-stimulus firing rate histograms (PSTHs) were generated in Matlab.Bins were set to 2 ms. Response latencies for cells with excitation were determined by finding the first bin which was at least 3σ greater than the baseline firing rate. Changes in the number of spikes fired and response types (excitation, inhibition or both) were calculated from regions of interest set in the average PSTH. An initial set of seed boundaries were set by the experimenter and boundaries were then narrowed by an automated script written in Matlab using a defined set of parameters. Brains showing an excitatory response started when the instantaneous firing rate was more than 3σ above the baseline firing rate and terminated when it declined below this threshold. Second, the start of inhibition was defined as a decrease in the firing rate 1σ below the baseline firing rate for at least 10 ms, and the end of inhibition was defined as when the firing rate returned to baseline.

Electrical and optogenetic cerebellar stimulations. To stimulate the cerebellar dentate nucleus, all mice were implanted with a stimulating electrode or a fiber optic implant contralateral to the basal ganglia recording side. At least 200 trials per intensity were obtained with both electrical and optogenetic stimulations.

For electrical stimulation, a twisted bipolar electrode (PlasticsOne, +130-kΩ impedance) was implanted using the coordinates: ML −2.3 mm; AP −6.00 mm; 2.4 mm from the surface of the brain. Electrical stimuli were delivered as 200-µs constant current or voltage pulses ranging from 1–10 V or 10–100 µA in all cases, the strength of electrical stimulations are reported in terms of current taking into consideration the impedance of the stimulation electrodes). To optogenetically excite cells, we used an AVV vector to express the light-sensitive cation channel channelrhodopsin in the target cells and then as needed activated the channels by exposing them to light of the appropriate wavelength. To optically stimulate the dentate nucleus, 1 µl of AAV2-Syn-ChrR2(H134R)-YFP (University of North Carolina Vector Core) or 0.7 µl of AAV2/1-Syn-ChrR2(H134R)-YFP (University of Pennsylvania Vector Core) was injected into the same coordinates at a rate of 0.1 µl min−1. An optical fiber (200 µm in diameter, 0.48 NA, Thorlabs) was subsequently implanted in the injection site to deliver light during the experiment. The exact location where ChrR2 was expressed, and the position of the fiber optic was ascertained histologically. To visualize expression of ChrR2 and ArchT, tissue was stained with rabbit antibody to GFP (1:250 dilution, Molecular Probes by Life Technologies, Cat. No. A11122) conjugated with Alexa Fluor 488 and counter stained with Hoechst 33342 (1:400 dilution. Molecular Probes). Postmortem GFP staining and fluorescence microscopy confirmed the expression and appropriate selective targeting of ChrR2 to the dentate nucleus (see Supplementary Fig. 1c). Optical stimuli were delivered as 10-ms pulses using a 450-nm or 473-nm laser (OEM Laser Systems). Reported light powers correspond to the light intensity at the tip of the fiber optic exposed to tissue. Stimuli ranged from 0.5–5 mW.

Three sets of experiments were done to ascertain that optogenetic cerebellar stimulations activated cerebellar nuclei neurons by activating ChrR2. First, ChrR2 takes several weeks to optimally get expressed in neurons, and in all animals examined stimulations at maximum intensity did not produce a response for at least 10 d post-surgery, suggesting that it was ChrR2 expression, rather than non-specific actions of light in the cerebellum that mediated the responses. Second, strong optical stimulations (>10 mW) using a 640 nm that does not activate ChrR2 never produced a response whereas much lower intensity stimulations using the appropriate 450- or 473-nm lasers were very effective. Third, single-unit recordings were made in a head-restrained, awake mouse expressing ChrR2 in the dentate nucleus of the cerebellum using a custom-made optrode. Brief (10 ms) pulses of light reliably increased the firing rate of the neuron recorded (Supplementary Fig. 1d).

Inactivation of the thalamus. To chemically inactivate the thalamus with the sodium channel blockers TTX or QX314, we used previously published set of injection parameters that ensure selective, localized perfusion of brain structures31. Briefly, a subset of mice were implanted with a 26 gauge guide cannula (Small Parts) targeting the centrolateral nucleus of the thalamus (AP −1.58 mm; ML −0.8 mm; 3 mm from the brain’s surface; ipsilateral to the recording site). An acute injector (Plastics One) connected to an automated pump was placed in the guide cannula and 1 µl of 100 mM QX-314 or 50 mM tetrodotoxin (Tocris) was infused through the injector at a rate of 0.2 µl min−1. Typically, attenuation of cerebellar responses began 20 min after infusion onset, remained effective for at least 30 min and washed out about 1 h later.

In a few mice, the intralaminar nuclei were silenced optogenetically by expressing the light sensitive proton transporter archaerhodopsin in the target cells. To do so, 1 µl AV2/2-CAG-ArchT-GFP (University of North Carolina Vector Core) was injected in the same coordinates delineated above at a rate of 0.1 µl min−1. An optical fiber (200 µm in diameter, 0.48 NA, Thorlabs) was then implanted targeting CL. Optical inhibition of the thalamus was achieved using a 640-nm laser (OEM Laser Systems) with a power of 5–10 mW. The laser was turned on 500 ms before cerebellar stimulation and turned off 500 ms after. Postmortem GFP staining and fluorescence microscopy was used to confirm the expression and appropriate targeting of ArchT to the intralaminar thalamic nuclei in addition to the position of the fiber optic in the structure.

Silencing of the motor cortex. To chemically inactivate the motor cortex, two stainless steel 26 gauge cannulas (Small Parts) were implanted in the cortex (ML 1.5 mm; AP 1.55/−0.55 mm; 0.5 mm from brain surface). 2–3 V (set at the threshold for eliciting overt movements), 200–µs pulses were applied to the cannulas. Once a striatal response was observed, 3 µl of 50 mM TTX or 100 mM QX-314 was infused at 0.2 µl min−1 through the cannula which had produced the largest response in the target striatal neuron. Typically within 20–30 min of the onset of infusion of the blocker the firing rate of the target striatal neuron decreased. To surgically remove the motor cortex, a large section of the skull was removed and the cortex was aspirated. Four points marking the corners of a rectangle were used to identify the area for aspiration. They were: ML ± 2 mm, AP 2.5 mm and ML ± 1 mm, AP −1 mm. During aspiration, the white matter tracts of the corpus callosum were used to reference the depth of ablation and used as a lower limit for the aspirator (~1 mm). The cavity was then filled with gel foam for recovery. Recordings were made from these mice 3 weeks after removal of the cortex.

Stimulation of the motor cortex and cortico-striatal plasticity. To stimulate the motor cortex, a metal tube (14 gauge, Small Parts) was integrated into the bottom edge of the microdrive containing the microwire array. This metal tube was implanted 0.5 mm into the motor cortex and used for stimulation. To test
striatal responses to cortical stimulation, 200-µs voltage pulses ranging from 1–4 V were used. These intensities corresponded to 6–22 µA, respectively. To confirm that electrodes were implanted in the motor cortex and to determine the maximum stimulus intensity, stimulus intensity was gradually increased until they elicited movements. The highest intensity that did not illicit movements was used to probe cortico-striatal inputs and was subsequently also used for plasticity induction.

The HFS protocol used to induce cortico-striatal plasticity consisted of a train of 100-Hz pulses for 1 s, repeated four times with 10-s intervals between the trains. This was delivered to the motor cortex as described above. Only striatal cells that showed an excitatory response to cortical stimulation or excitation followed by inhibition were considered for analysis, although only the effect of the plasticity procedure on the excitatory component was quantified.

To test whether the weakening of the cortico-striatal response following HFS was dependent on endocannabinoids, we administered AM-251 at least 30 min before starting experiments. AM-251 was dissolved in 20% DMSO and 80% distilled water and injected at 5 mg per kg. Experiments continued as discussed above. To potentiate cortico-striatal responses, in addition to the motor cortex, the cerebellum was also stimulated with a high-frequency train, although cerebellar stimulations were performed optogenetically. Cortical stimulation trains were delivered as described above. Cerebellar HFS consisted of a train of 50-Hz light pulses for 1 s, repeated four times with an interval of 10 s. Thus, both the cerebellar and cortical trains occurred concurrently. Two criteria had to be satisfied before start of such a plasticity protocol. First, the striatal neuron’s response to cerebellar stimulation had to be excitatory. Second, the striatal neuron needed to show a sustained increase in its firing rate when the cerebellum was excited with five pulses at 50 Hz (Fig. 7).

fEMG and striatal recordings in mice with cerebellar-induced dystonia. To examine the role of cerebellar-basal ganglia interactions in cerebellar-induced dystonia, we used a previously established mouse model of rapid onset Dystonia Parkinsonism, which is caused by loss-of-function mutations in the sodium pump. In this mouse model, acute or chronic perfusion of ouabain, a selective blocker of sodium pumps, into the cerebellum is sufficient to cause dystonia31. In a single surgery, a cannula was implanted into the cerebellum and a microwire array was implanted into the dorsolateral striatum. Once baseline recordings were made from dorsolateral striatal neurons as described, dystonia was acutely induced by infusing ouabain (100 µM) into the cerebellum via the cannula at a rate of 0.3 µl min⁻¹ following previously published procedures31. This allowed for direct comparison of the activity of some cells before and after induction of dystonia.

To monitor the time of onset of dystonic postures, we also performed fEMG recordings31 by inserting a wire under the skin on the back of the animal approximately half way between the neck and the tail. The position of the recording electrodes and the perfusion cannula were ascertained postmortem by histology as described earlier.

Alleviation of cerebellar-induced dystonia by electrical lesioning of CL. It is already established that bilaterally lesioning CL prevents induction of cerebellar-induced dystonia31. We explored whether electrical lesion of CL can alleviate previously generated cerebellar-induced dystonia. To be able to follow dystonic animals for several days after electrically lesioning CL we generated the long-lasting form of dystonia by chronically perfusing ouabain into the cerebellum as previously described31. A single cannula was stereotaxically implanted at the cerebellar midline (AP, −6.90 mm from bregma; DV, 2 mm) and connected to an osmotic pump (0.25 µl h⁻¹, Alzet) containing ouabain (100 µM) and 0.01% methylene blue (wt/vol), which allowed for post-mortem examination of the perfusion site. The pump was then placed under the skin on the back of the mice. With this procedure, mice showed dystonia within 24 h after the surgery.

To be able to lesion the CL once dystonia was induced, in the same surgery custom made electrodes were bilaterally implanted into CL (AP, −1.58 mm from Bregma; DV, 3.5 mm; ML, 0.8 mm and −0.8 mm). After onset of dystonia, lesions were made using 300-µA, 45-s-long current pulses at each CL site. Given that the disynaptic pathway passes through the posterior two-thirds of CL in rodents, we targeted the posterior portion of this nucleus. The extent and location of lesions were determined histologically using Nissl staining as reported previously31. Only mice in which the specificity of CL lesions was histologically confirmed were included in the analysis. The efficacy of the CL lesions in alleviating dystonia was evaluated 24 h after the lesion.

Alleviation of cerebellar-induced dystonia by optogenetic inactivation of the CL. The intralaminar nuclei and primarily the CL were optogenetically silenced and the effect on cerebellar-induced dystonia examined. In a single surgery, 1 µl AAV2/5-CAG-ArchT-GFP (University of North Carolina Vector Core) was injected into CL (AP, −1.58 mm from Bregma; DV, 3.5 mm; ML, 0.8 mm and −0.8 mm), fiber optics were implanted immediately above the injection site to target the CL, and an infusion cannula was implanted into the cerebellum (AP: −6.90 mm from bregma; DV: 2 mm). After 2 weeks for opsin expression, dystonia was induced by infusing ouabain (100 µM) into the cerebellum via the cannula at a rate of 0.3 µl min⁻¹ as described earlier. Once dystonia manifested, the intralaminar nuclei were silenced by activating a 640-nm laser (OEM Laser Systems) with a power of 5–10 mW.

Dystonia score. The presence of dystonia and its severity were quantified using a previously published scale31. Briefly, 0 = normal behavior; 1 = abnormal motor behavior, no dystonic postures; 2 = mild motor impairment, dystonic-like postures when disturbed; 3 = moderate impairment, frequent spontaneous dystonic postures; 4 = severe impairment, sustained dystonic postures. The assessment of dystonia was made independently by three trained observers blinded to the condition of the mice. All three observers scored the same video clips. Their scores were averaged by the authors.

Statistical analyses. We examined the variance in each group with the F test and determined whether the data exhibited a Gaussian distribution with the D’Agostino-Pearson omnibus normality test. We analyzed the data using parametric (two-samples, one/two-tailed Student’s t test or ANOVA) or non-parametric (one/two-tailed Mann-Whitney U, Kruskal-Wallis, paired or one sample Wilcoxon signed ranks) depending on whether the data displayed or did not display a Gaussian distribution, equal variance or similar sample size. We compared the observed proportion of distributions using the chi-square test for goodness of fit. To estimate ideal sample sizes, power analyses were conducted using the first few experiments to determine mean and s.d. based on the magnitude of the anticipated effect (power 0.90 with a significance value of 0.05). Sample sizes for experiments alleviating dystonia were determined from previously published experiments. All experiments met or exceeded ideal sample sizes.

A Supplementary Methods Checklist is available.