Neuromodulation of the cerebellum rescues movement in a mouse model of ataxia

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Deep brain stimulation (DBS) relieves motor dysfunction in Parkinson's disease, and other movement disorders. Here, we demonstrate the potential benefits of DBS in a model of ataxia by targeting the cerebellum, a major motor center in the brain. We use the Car8 mouse model of hereditary ataxia to test the potential of using cerebellar nuclei DBS plus physical activity to restore movement. While low-frequency cerebellar DBS alone improves Car8 mobility and muscle function, adding skilled exercise to the treatment regimen additionally rescues limb coordination and stepping. Importantly, the gains persist in the absence of further stimulation. Because DBS promotes the most dramatic improvements in mice with early-stage ataxia, we postulated that cerebellar circuit function affects stimulation efficacy. Indeed, genetically eliminating Purkinje cell neurotransmission blocked the ability of DBS to reduce ataxia. These findings may be valuable in devising future DBS strategies.

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Deep brain stimulation (DBS) is an FDA-approved, neurosurgical technique used to treat motor and non-motor diseases including Parkinson’s disease, dystonia, tremor, and obsessive–compulsive disorder. DBS strategies have targeted the thalamus, globus pallidus, subthalamic nucleus, and nucleus accumbens. DBS benefits depend on the parameters used and on the symptoms being treated. For instance, in dystonia, DBS can be ~85% or <20% effective. While technical challenges such as electrode drift impact patient responsiveness, other problems such as motor decline and tolerance suggest suboptimal brain targeting. Indeed, stimulating different targets improves medically-refractory dystonia. We hypothesize that stimulating the motor regions that contribute most to key symptoms at the time of treatment could improve behavioral outcomes.

To test this hypothesis, we targeted DBS to the cerebellum, a primary motor control center, and asked whether stimulation restores movement in ataxia. The choice of the cerebellum as the target, and ataxia as the symptom, was motivated by several reasons. First, cerebellar outflow circuits have major therapeutic potential since they connect the cerebellum with more than three dozen brain and spinal cord nuclei. Critical computational sites in the cerebellar circuit are also experimentally accessible and highly plastic, which, in theory, could support long-term, behavioral improvements. Moreover, the cerebellum plays a pathogenic role in a growing list of ataxias, including spinocerebellar ataxia, Friedrich’s ataxia, ataxia telangiectasia, and episodic ataxia—all of which are characterized by a progressive decline in motor function and eventually, immobility. Contrary to the sometimes variable effects of improving basal ganglia function to reduce dystonia, improving cerebellar function universally recovers mobility in different ataxias, partly because the cerebellum is distinctly vulnerable to hereditary and non-hereditary insults. Illustratively, the principle cell type of the cerebellar circuit, the Purkinje cell, selectively degenerates in most forms of ataxia, as do the downstream cerebellar and brainstem nuclei. Accordingly, a major goal of ataxia research has been to elucidate the molecular mechanisms underlying this specificity, which could then be used to tailor pharmaceuticals and improve the efficacy of gene and stem cell therapies. However, there are many practical challenges to applying these therapies in vivo, including efficient delivery of the agent to a large number of affected neurons, correcting complex cellular functions and animal behaviors, and maintaining the benefits throughout life.

We argue that DBS targeted to the cerebellum bypasses several critical hurdles. While stimulating the cerebellar cortex was inconsistent for treating motor deficits, stimulating the cerebellar nuclei has been more efficacious (EDEN; Improvement of Upper Extremity Hemiparesis Due to Ischemic Stroke: A Safety and Feasibility Study, ClinicalTrials.gov Identifier: NCT02835443). Here, we investigated whether DBS combined with physical activity enhances motor function in the Car8 waddles (Car8w) mutant mouse that models a form of hereditary ataxia in humans. Our study was motivated by the presence of carryover effects observed after DBS is terminated in patients. We therefore asked whether such carryover effects can be controlled in a manner that would provide deliberate, long-term benefits to behavior. Particularly valuable to our study is that cerebellar circuit function is altered in Car8w mice, but the cells do not develop overt anatomical defects and they do not degenerate. Therefore, cerebellar neural circuitry is experimentally accessible and amenable to treatment and post-stimulation tracking. Our approach tests the therapeutic potential of the cerebellum by targeting DBS to the interposed nuclei. We show that cerebellar DBS restores motion in ataxia and that the rescue of motor behavior was the greatest when the treatment is paired with exercise and starts early after the onset of ataxia.

**Results**

**Beta-frequency DBS into the cerebellum improves Car8w motor behavior.** We recently developed a DBS strategy that targets the interposed cerebellar nucleus, which sends major connections to the red nucleus and thalamus to control ongoing motor function. Unlike the dentate or fastigial nucleus, the interposed nucleus facilitates locomotor adaptation and contains cerebellosensory neurons, which synchronically target pre-motor circuits in the spinal cord. Feedback collaterals from the red nucleus also selectively innervate the interposed nucleus, suggesting that the interposed nucleus can independently process sensorimotor information and influence corollary discharge. Since balance, coordination, locomotor adaptation, and sensorimotor processing degrade in ataxia, we reasoned that the interposed nucleus could be an ideal target for restoring movement.

The impact of DBS specifically on gait in ataxia, as demonstrated in a recent study, additionally motivated our design of a therapeutic approach that might boost its efficacy. This included stimulating the interposed nuclei of ataxic Car8w mice (Fig. 1a–d; Supplementary Figs. 1–2) while the mice were actively engaged on an accelerating rotarod. In this paradigm, the rotarod assay serves as a proxy for challenging exercise, since it is a demanding physical task that also requires skilled learning. We pair cerebellar stimulation with exercise because previous attempts to restore function in Parkinson’s disease and stroke show that combining neuromodulation with motor rehabilitation significantly enhances therapeutic outcomes. However, this combinatorial approach has only been tested in severe neurodegenerative diseases, where plastic changes in surviving circuits, such as compensatory miswiring after axonal regression and significant alterations in dendritic architecture, occur. It is unclear if additional therapeutic benefits could be unlocked for ataxia if physical activity is combined with DBS when circuits are dysfunctional, but anatomically intact, or when DBS is targeted to a cerebellar region that contains circuitry that is specifically involved in sensorimotor processing and locomotor adaptation during ongoing, intentional movements.

Our behavioral paradigm consisted of two periods: “Before ±DBS” and “With or Without DBS” (Fig. 1a, b). We measured the latency to fall off of the accelerating rotarod for Car8w mice during both of these periods to calculate their improvement during stimulation at various frequencies. We specifically stimulated the cerebellar nuclei at 0, 2, 13, and 130 Hz (Fig. 1e, f; Supplementary Fig. 1), given that these frequencies support distinct aspects of normal circuit function. For instance, delta- and theta-frequencies between 1 and 9 Hz promote cerebellar learning at climbing fiber-to-Purkinje cell as well as parallel fiber-to-Purkinje cell synapses. In contrast, beta-frequencies from 10 to 30 Hz facilitate communication in the cerebellar-thalamo-cortical pathway, and higher frequencies between 30 and 260 Hz promote scaling, planning, and neural synchronization.

We found that Car8w mice improve on the rotarod with cerebellar DBS, but only when it is delivered at a beta-frequency (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095). We conducted several control experiments to substantiate these effects. Without surgery, Car8w mutant mice do not significantly improve (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095). We conducted several control experiments to substantiate these effects. Without surgery, Car8w mutant mice do not significantly improve (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095). We conducted several control experiments to substantiate these effects. Without surgery, Car8w mutant mice do not significantly improve (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095). We conducted several control experiments to substantiate these effects. Without surgery, Car8w mutant mice do not significantly improve (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095). We conducted several control experiments to substantiate these effects. Without surgery, Car8w mutant mice do not significantly improve (Fig. 1e, f; Supplementary Fig. 1c; Supplementary Movie 1; 13 Hz, 65.29 ± 25.11%, n = 12, p = 0.0095).
Supplementary Movie 3; No Surgery, 6.58 ± 2.01%, n = 10, p = 0.9463; 0 Hz, 20.43 ± 7.00%, n = 15, p = 0.0321; 2 Hz, 28.94 ± 9.57%, n = 6, p = 0.0166; 13 Hz, 12.12 ± 8.08%, n = 4, p = 0.8414; 130 Hz, 33.79 ± 8.34%, n = 11, p < 0.0001). These data show that our cerebellar DBS approach improves motor behavior in Car8wdd mice, particularly at a frequency that normally supports proper neural communication of cerebellar output signals.

Surgical lesions caused by DBS electrodes do not compromise motor circuit integrity. Cerebellar stimulation has long-distance effects on the motor circuit through modulating cortical excitability33 and corollary discharge30. If stimulating the interposed nucleus promotes communication between the cerebellum and the cortex or muscles to improve locomotion, then circuit anatomy should be intact. Therefore, we survey the motor circuit for damage using whole-mount imaging, histology, and anterograde wheat-germ agglutinin (WGA)-Alexa 555 tracing from the rostral cerebellum (Supplementary Fig. 4)–6). While neurodegeneration eliminates key circuit components in the brain, contusions and hemorrhaging impair tissue metabolism and cause massive neuronal depolarization36,37. Moreover, glial scarring and inflammation impact neuronal output through stunting axonal regrowth and promoting cell loss, respectively38,39. Such effects could counteract cerebellar outflow communication. We found no bruising or hemorrhaging in the cerebellum (Supplementary Fig. 8; GFAP (0, 13, 130 Hz): Controls, n = 3 (each), 59.269 ± 6.947, 64.121 ± 5.783, 69.023 ± 7.465; Mutants, n = 3 (each), 66.543 ± 3.497, 62.647 ± 5.506, 83.444 ± 11.808, p > 0.9999 (0 Hz), p = 0.9556 (13 Hz); Iba1 (0, 13, 130 Hz): Controls, n = 3 (each), 24.609 ± 8.391, 38.690 ± 4.599, 36.339 ± 3.953; Mutants, n = 3 (each), 30.630 ± 2.954, 51.615 ± 11.363, 37.827 ± 1.256, p > 0.9999 (0 Hz), p = 0.9486 (13 Hz)). We also examined brain-muscle integrity by testing muscle responsiveness to descending neural modulation. We specifically examined the slow-twitch and fast-twitch fiber composition of the tibialis anterior (TA) because previous work demonstrated that it changes after exogenous stimulation40. We found that cerebellar stimulation increases slow-twitch fiber protein expression in both control and Car8wdd mice, although this change is only statistically significant in the mutant muscles (Supplementary Fig. 9; Controls: 0 Hz: n = 3, 10.83 ± 4.59%; 13 Hz: n = 3, 31.77 ± 8.73%; p = 0.0787; Mutants: 0 Hz: n = 3, 22.89 ± 11.13%; 13 Hz: n = 3, 47.55 ± 6.22%; p = 0.0312). These data show that the Car8 mutation does not block muscle responsiveness to descending neural modulation. Altogether, we deduce that the surgical lesions created after implanting the DBS electrodes do not impede cerebello-thalamo-cortical communication or the integrity of neuromuscular connectivity. These intact, motor connections may promote functional recovery, which is a key benefit of stimulating Car8wdd mice.

Cerebellar DBS normalizes Car8wdd muscle activity during locomotion. When the cerebello-thalamo-cortical circuit is engaged during locomotion, the muscles are activated in a temporally precise manner41. Although direct, exogenous stimulation improves the force, resistance, and length of muscle contractions, it is unclear if neuromodulation alters motor timing to improve...
locomotion. To test this, we measured electromyography (EMG) activity in active Car8wdl mutant mice (Fig. 2a, b). We previously showed that Car8wdl mice have prolonged contractions of the tibialis anterior (TA) muscle. The TA is important for locomotion because this task requires its activity to fire out-of-phase with the activity of the gastrocnemius muscle (GC; Fig. 2c, d). When the activities of the TA and GC muscles overlap, co-contractions occur, and locomotion is impaired (Fig. 2d). In Car8wdl mice, prolonged TA firing likely contributes to their locomotor deficits by increasing the probability that their hindlimb muscles co-contract (Fig. 2d). However, this abnormal muscle phenotype is rescued in Car8wdl mice, when EMG is combined with 13 Hz cerebellar DBS (Fig. 2e; Supplementary Fig. 6; 0 Hz: 0.817 ± 0.357 bursts/ms). Furthermore, 13 Hz DBS significantly shortens the muscle contraction time of Car8wdl mice (p = 0.0005). n = 24 biologically independent animals (Control: 0 Hz, n = 4, 13 Hz, n = 8; Car8wdl: 0 Hz, n = 6, 13 Hz, n = 6) over 24 independent experiments. ***p < 0.001; three-way ANOVA, repeated measures; Sidak’s multiple comparisons test; mean ± SEM.

Success of low-frequency cerebellar DBS depends on multiple motor behavior responses. We next tested how neuromodulation impacts mobility during different motor tasks. We measured the effects of DBS on the general locomotion of Car8wdl mice by using the open field assay, and we studied the repercussions of stopping DBS on muscle activity and motor learning, through using EMG and the accelerating rotarod, respectively. When combining cerebellar DBS with the open field assay, we found that only 13 Hz prevents motor degradation after surgery in Car8wdl mice (Fig. 3a, b). Supplementary Fig. 11; No Surgery, n = 3, Day 0, 1276.00 ± 141.37 s versus Day 13, 1162.00 ± 157.77 s, p = 0.7089; 0 Hz, n = 5; Day 0, 1255.66 ± 147.55 s versus Day 13, 793.26 ± 109.66 s, p = 0.0017; 13 Hz: n = 3, Day 0, 1297.87 ± 78.92 s versus Day 13, 1268.90 ± 19.77 s, p = 0.9771; No Surgery/13 Hz, Day 13, p = 0.5382). Because 13 Hz DBS had the most
beneficial effects on muscle function (Fig. 2) and the least negative impact on freely moving behavior (Fig. 3a, b), these data might explain why only 13-Hz stimulated Car8wdl mice improve their performances on the rotarod (Fig. 1).

To test whether 13 Hz DBS promotes locomotion by normalizing Car8wdl muscle function, we measured muscle activity after stopping DBS (Fig. 3c). Interestingly, we found that Car8wdl TA function remains corrected (Fig. 3c; \( n = 6; 123.79 \pm 28.80 \) s; Fig. 3 Cerebellar DBS promotes locomotor improvements for ongoing motor function. a Schematic and experimental timeline of the open field assay (OFA) used in combination with DBS to measure movement and movement time. Example traces reveal that 13 Hz DBS maintains the movement of Car8wdl mice. b No other stimulation frequency other than 13 Hz preserves the movement of Car8wdl mice. Schematic summarizes the effects of cerebellar stimulation on Car8wdl movement and compares how alterations cause Car8wdl behavior to deviate from that of controls. The darker the gray, the more stimulation impacted Car8wdl movement. The wider the schematic, the more Car8wdl motor behavior deviated from control motor behavior (black dotted vertical line). The schematic aligns with the stimulation frequencies on the left. c TA muscle firing in Car8wdl mice remains reduced after stopping DBS (Day 1: \( p = 0.0013; \) Day 3: \( p = 0.0492 \)). Example traces from a 13-Hz stimulated Car8wdl mouse are outlined. Before = Before ±DBS, Day 1; 1 = After ±DBS, Day 1; 3 = After ±DBS, Day 3. \( n = 24 \) biologically independent animals (Control, 0 Hz: \( n = 4; \) Control, 13 Hz: \( n = 8; \) Car8wdl, 0 Hz: \( n = 6; \) Car8wdl, 13 Hz: \( n = 6 \)) over 24 independent experiments on Day 1. \( n = 15 \) biologically independent animals (Control, 0 Hz: \( n = 3; \) Control, 13 Hz: \( n = 3; \) Car8wdl, 0 Hz: \( n = 4; \) Car8wdl, 13 Hz: \( n = 5 \)) over 15 independent experiments on Day 3. d Experimental timeline and schematic of our unpaired behavioral paradigm. The mutants perform on the rotarod without DBS. e Car8wdl mice need to be stimulated while on the rotarod to receive benefits (\( p = 0.9999 \)). The black dotted line demarcates the mutant baseline (normalized to \( y = 1.00 \)). The latency to fall values for each Car8wdl mouse were plotted, before and during unpaired DBS. \( n = 24 \) biologically independent animals (No Surgery: \( n = 8; \) 0 Hz: \( n = 12; \) unpaired 13 Hz: \( n = 4 \)) over 24 independent experiments. * \( p < 0.05; \) ** \( p < 0.01; \) three-way ANOVA, repeated measures with mixed-effects (c); two-way ANOVA, repeated measures (e); Sidak’s multiple comparisons test (c, e); mean ± SEM.
Before/After, \( p = 0.0013 \). Stimulating the cerebellum daily, as performed in the rotarod experiments, further suppresses TA pathology (Supplementary Fig. 12; \( n = 5 \); 102.32 ± 19.27 ms; Day 1/Day 3, \( p = 0.0492 \). If normalized muscle function alone mediates improvements, then we would expect Car8wdl mice to achieve the same benefits, even when DBS is no longer being delivered during the behavior. To test this possibility, we stimulated Car8wdl mice, then terminated the DBS right before placing the mice onto the rotarod (Fig. 3d). Car8wdl mice did not improve using this paradigm (Fig. 3e; 13 Hz, unpaired: \( n = 4 \), \(-4.39 ± 31.42 \% \), \( p = 0.9999 \)), which suggests that the ideal context for improving complex motor behaviors, such as motor coordination, is the case with Car8wdl mice requiring 13 Hz DBS to engage the motor function machinery that is actively operating at the time of the stimulation.

Cerebellar DBS induces long-lasting motor benefits in Car8wdl mice. One way in which ongoing learning manifests is through gait adaptations. By combining DBS with footprint and DigiGait analyses, we found that 13 Hz DBS alters the step cycle, but not the overall gait of Car8wdl mice (Fig. 4a–e; Supplementary Fig. 13). A step cycle consists of braking, propulsion, and a swing phase. The paw contacts the ground during “braking” and “propulsion,” but not during the “swing” (Fig. 4b). The mice experience the greatest stability while braking. With reference to these measures, Car8wdl mice spend significantly less time braking (\( n = 21 \); 0.094 ± 0.004 s) than do control mice (Fig. 4c; \( n = 19 \), 0.110 ± 0.004 s, \( p = 0.0037 \)), which corroborate our previous findings that the paws of Car8wdl mice contact the ground less during locomotion than do the paws of control mice.25 However, 13 Hz DBS prolongs the braking of Car8wdl hindlimbs (Fig. 4d; Day 13; 0 Hz, \( n = 5 \), 0.093 ± 0.010 s, \( p = 0.9498 \); 13 Hz, \( n = 4 \), 0.110 ± 0.002 s, \( p = 0.00307 \)). Interestingly, the response is sustained 7 days after stimulation stops (Fig. 4e; Day 20; 0 Hz, \( n = 5 \), 0.082 ± 0.003 s, \( p = 0.9484 \); 13 Hz, \( n = 4 \), 0.114 ± 0.013 s, \( p = 0.1034 \)). Compared to control mice (\( n = 4 \)), these results reveal that DBS corrects the motor step cycle (Fig. 4e; Supplementary Data 1; Day 0: 0.127 ± 0.008 s; Day 20: 0.124 ± 0.006 s, \( p = 0.9993 \); Control/Mutant (±13 Hz): Day 0, p = 0.0076 versus Day 20, p = 0.8727) by stabilizing ongoing movements in Car8wdl mice, during and after stimulation.

To investigate whether other motor outcomes are retained, we tracked the movement of Car8wdl mice and measured their coordination, 4–7 days after DBS was introduced (Fig. 4f–h; Rotarod, Days 15–18, Open field, Day 20). We found that previously stimulated Car8wdl mice continue to exhibit increased locomotion (Fig. 4g, h; No Surgery, \( n = 3 \); Day 20, 960.63 ± 131.53 s, \( p = 0.1098 \); 0 Hz, \( n = 5 \); Day 20, 892.74 ± 129.93 s, \( p = 0.0134 \); 13 Hz: \( n = 3 \); Day 20, 975.43 ± 125.77 s, \( p = 0.1008 \) and they show improvements on the rotarod, starting 4 days after stimulation stops (Fig. 4f; Supplementary Movie 4; No Surgery, \( n = 8 \), 33.62 ± 21.85%, \( p = 0.6413 \); 0 Hz, \( n = 5 \), 31.01 ± 34.26%, \( p = 0.4267 \); 13 Hz, \( n = 12 \), 120.14 ± 34.12%, \( p = 0.0053 \)). These results underscore the long-term benefits of providing 13 Hz cerebellar DBS to restore mobility in Car8wdl mutant mice.

Ataxia severity contributes to the variability of DBS outcomes. We show that stimulating cerebellar outputs has therapeutic value, but the benefits are variable, as reported in humans.44,45 Besides inefficient electrode targeting46 and insertional damage47, inappropriate stimulus parameters48 and suboptimal electrode design and impedance49,50 also impact DBS effectiveness. Despite efforts to reduce the impact of these factors on patient outcomes,51–53 responses still vary due to differences in age54 and disease severity55. A retrospective analysis of our data revealed that 58.3% (\( n = 7/12 \)) of the Car8wdl mice responded favorably to 13 Hz DBS (Fig. 5a; we defined mice as “responders” to DBS if they improved more than that of sham mice (39.76 ± 13.66%) on the rotarod during stimulation). A previous publication showed that a range of beta-frequencies (10, 20, and 30 Hz) comparably improves the fall rate of rodents.1 However, higher beta-frequencies (20–30 Hz) more effectively suppress incoordination by enhancing cerebello-thalamo-cortical communication31,35,56. We therefore tested whether increasing the stimulation frequency to 20 Hz would improve DBS responses, but found that the same number of Car8wdl mice improve with 20 Hz DBS (57.1%; \( n = 8/14 \)) and with equal robustness (Supplementary Fig. 14; \( p = 0.5660 \)), as compared to 13 Hz DBS. This shows that multiple beta-frequencies are effective at improving motor coordination in Car8wdl mice. Moreover, the data indicate that the therapeutic benefits of cerebellar DBS encompass an optimal range of frequencies rather than a single frequency. We therefore predict that there is flexibility in what might be considered an optimal therapy, which depends on each individual’s inherent circuit properties.

Another possibility for the observed variability may be symptom severity before stimulation. Indeed, when we plotted the latency to fall values for the mutant responders (\( n = 7 \); 13 Hz; \( n = 8 \), 20 Hz), we observed that stimulated Car8wdl mice performed the best when their ataxia was less severe (Fig. 5b; Supplementary Fig. 14c). If ataxia severity impacts responsiveness (measured as motor improvements on the rotarod), then DBS should improve motor function in Car8wdl mice whose ataxia has not progressed too far beyond the onset of symptoms at the time of stimulation. As hypothesized, we found that beta-frequency DBS was therapeutic for Car8wdl mice with less severe ataxia (Fig. 5c; P30; 0 Hz: \( n = 4 \), 47.15 ± 9.97%, \( p = 0.0840 \); 13 Hz: \( n = 4 \), 130.05 ± 53.21%, \( p = 0.0076 \)) and DBS did not worsen movement or gait (Supplementary Fig. 15; Supplementary Data 1). Importantly, we found that stimulating Car8wdl mice at the peak of their ataxia was not beneficial (Fig. 5d; Supplementary Fig. 16; ≥P150; Rotarod, 0 Hz: \( n = 6 \), 114.23 ± 42.80%, \( p = 0.2980 \); 13 Hz: \( n = 5 \), 87.20 ± 82.24%, \( p = 0.5557 \); EMG, \( n = 3 \), 96.39 ± 33.92 ms (Before DBS); 180.24 ± 51.89 ms (+13 Hz), \( p = 0.4208 \); 261.49 ± 66.94 ms (After DBS), \( p = 0.0465 \)). Together, these data indicate that a positive behavioral outcome to cerebellar DBS partially depends on the severity of ataxia at the time when treatment is initiated.

Purkinje cell firing properties change with motor deterioration in Car8wdl mice. Given that Car8wdl responders continue to improve after stimulation stops and that ataxia severity restricts DBS efficacy, circuit properties may help set the therapeutic window. We hypothesized that Purkinje cell activity may specifically impact DBS effectiveness due to their roles in motor learning and ataxia in Car8wdl mice24,25. Although it is unclear which Purkinje cell properties cause ataxia, alterations in their firing regularity disrupt cerebellar nuclei output and motor function57,58. Contributing to the motor dysfunction of Car8wdl mice is erratically firing Purkinje cells (i.e., increased pausing, increased coefficient of variation, CV) with reduced spike time variability (i.e., decreased CV2)24. While some of these features (i.e., decreased CV2) manifest during development (≥P20), when ataxia starts25, others (i.e., increased pausing, increased CV) emerge in Car8wdl mice (P60–P90)24 when motor function worsens (Supplementary Fig. 2). If motor deficits correlate with the regularity of Purkinje cell firing, then alterations in pausing, CV, and CV2 may also impact DBS effectiveness. We therefore measured the regularity of Purkinje cell firing in Car8wdl mice that respond (P30) and do not respond (≥P150) to DBS, through performing in vivo extracellular recordings (Fig. 6a, b). Car8wdl...
Purkinje cells only fire more irregularly on the whole than controls when motor function has significantly deteriorated (Fig. 6c–f; P30 (pause percent, CV): Control, n = 25 cells from 4 mice, 23.78 ± 2.98%, 3.864 ± 0.337 versus Mutant, n = 14 cells from 3 mice, 29.65 ± 5.21%, 4.682 ± 0.576, p = 0.2981, p = 0.1969; ≥P150 (pause percent, CV): Control, n = 11 cells from 4 mice, 2.07 ± 1.07%, 0.757 ± 0.188 versus Mutant, n = 11 cells from 7 mice, 18.08 ± 4.13%, 4.597 ± 1.169, p = 0.0013, p = 0.0041). However, Purkinje cell firing is more regular during local spike trains in Car8<sup>wdl</sup> mice at P20–P90<sup>24,25</sup>, but not at ≥P150.
Cerebellar DBS relies on Purkinje cell neurotransmission. Because we found that Purkinje cell function, motor behavior, and DBS responsiveness are inextricably linked, we asked whether modulating Purkinje cell neurotransmission is vital for treating ataxia. Previous attempts at improving ataxia through the use of pharmacologically-based treatments reveal that the greatest therapeutic effects emerge after normalizing Purkinje cell function, regardless of the model or genotype studied\textsuperscript{10,24,62}. Therefore, we postulated that stimulating L7\textsuperscript{Cre}, Vgat\textsuperscript{lox/lox} mice\textsuperscript{63}, a second ataxia model which lacks Purkinje cell GABAergic neurotransmission (Fig. 6g), would result in no improvements. The rationale for using this model is that Purkinje cell output is selectively blocked, a manipulation that precisely targets functional circuit properties without inducing large-scale anatomical rearrangements or neurodegeneration\textsuperscript{64}. As hypothesized, cerebellar DBS did not restore proper motor function in L7\textsuperscript{Cre}, Vgat\textsuperscript{lox/lox} mice (Fig. 6h, i; No Surgery: n = 3, −1.66 ± 6.37%, p = 0.9989; 0 Hz: n = 5, 27.81 ± 25.55%, p > 0.9999; 20 Hz: n = 5, −4.52 ± 17.96%, p = 0.9927). Given that eliminating GABAergic neurotransmission from Purkinje cells alone produces diverse motor deficits, such as disequilibrium and postural problems in addition to incoordination, it may be that different modes of chemical or electrical manipulations would fail to elicit improvements for multiple cerebellar deficits. Indeed, we recently demonstrated that administering a tremorgenic drug to L7\textsuperscript{Cre}, Vgat\textsuperscript{lox/lox} mice does not alter motor behavior either\textsuperscript{27}. These results indicate that a closed-loop cerebellar circuit is needed to propagate or rescue motor dysfunction in ataxic mice. Therefore, the therapeutic signals provided by the cerebellar nuclei DBS could conceivably exit the cerebellum and then return to use the computational power of the Purkinje cells to mediate circuit repair, or the signals could retrogradely travel from the cerebellar nuclei back into the cerebellar cortex to more directly impact Purkinje cell function.
Discussion

We show that stimulating the Car8wdl cerebellum at 13 Hz results in short-term and long-term motor improvements. While neurostimulation improves muscle function (Fig. 2) and general mobility in Car8wdl mice (Fig. 3), improvements to motor coordination (Fig. 1) and stepping (Fig. 4) require DBS to continue during exercise (Fig. 3). These data highlight the importance of treatment design for DBS effectiveness: not only does electrode
targeting matter, but so does delivery. Since coordinative treadmill training improves ataxia64,65, as well as Purkinje cell and muscle health66–68, we hypothesize that supplementing DBS with skilled exercise improves cerebellar learning and muscle function in order to facilitate retention and enhance motor recovery.

We provide several lines of evidence showing that DBS improves cerebellar and muscle functions, including that stimulation improves motor coordination and muscle function during movement (Figs. 1 and 2; Supplementary Movie 1). These results can partly be attributed to our targeting of the interposed cerebellar nuclei (Supplementary Fig. 1). The interposed cerebellar nuclei communicate with neurons in the thalamus, red nucleus, and spinal cord to control ongoing movements. On the local circuit level, the interposed nucleus facilitates motor learning6. Although we cannot rule out the possibility that the dentate and fastigial nuclei are also stimulated in our paradigm given the relatively small size of the interposed, our rotarod, DigiGait, and EMG findings support predominant interposed stimulation for at least two reasons: sensorimotor feedback likely contributes to Car8well motor improvements and locomotor adaptation occurs. The interposed nucleus specifically receives sensory information from spinal circuits to modulate locomotion28. In our study, cerebellar DBS improves complex motor behaviors in Car8well mice only when it is paired with tasks that generate abundant sensorimotor feedback, such as the rotarod (Fig. 3). Our EMG data also corroborate the role of sensorimotor feedback in mediating DBS responses. Not only does sensory feedback modulate the EMG activity of locomotor muscles59, it also alters motor function in a predictable manner: first temporally, then spatially28. In our study, we found that DBS improves TA firing in as little as 20 min (Fig. 2), but behavioral modifications occur anywhere from 2 h (Fig. 3) to 3–18 days later (Fig. 4; Supplementary Figs. 11 and 14).

Besides reinforcing our targeting, our behavioral data provides insight into how neuromodulation might permanently restore locomotion in vivo. With regards to muscle function, our open field and EMG results support the possibility of DBS altering the function of multiple muscle groups. Here, we studied the TA given its dysfunction in function of multiple muscle groups. Here, we studied the TA (Supplementary Fig. 14), but not with higher frequencies (Supplementary Fig. 1). Our finding that control mice benefit from 0, 2, and 130 Hz, but not 13 Hz, reveals that there may be a ceiling effect for how much control mice can improve (Supplementary Fig. 3). This is evidenced by our histology data, which shows comparable changes to muscle composition between the control and Car8well mice after providing 13 Hz DBS (Supplementary Fig. 9; 13-Hz stimulated control compared to 13-Hz stimulated Car8well; Slow: $p = 0.5813$), and our rotarod data, which shows that the motor improvements in control mice depend on an initial presence of behavioral deficits—e.g., due to implant lesions. For example, the control mice with significantly worse (0 and 130 Hz) or highly variable (2 Hz) starts, are the groups that improve with DBS (Supplementary Fig. 3; No Surgery, $n = 10$, 267.313 ± 5.601 s; 0 Hz, $n = 15$, 194.358 ± 15.628 s; 2 Hz, $n = 6$, 200.757 ± 18.544 s; 130 Hz, $n = 11$, 187.591 ± 15.147 s; No/0 Hz, $p = 0.0076$; No/2 Hz, $p = 0.1410$; No/130 Hz, $p = 0.0058$). After DBS has been initiated, 0, 2, and 130-Hz stimulated control mice perform equally as well on the rotarod as any other group.

Perhaps most insightful toward understanding how DBS works in vivo are the long-lasting benefits that Car8well mice receive after stimulation (Figs. 3–5), as this suggests that carryover effects are maintained by our motor function circuitry. In humans, carryover effects have been reported after stimulating the basal ganglia in dystonia and Parkinson’s disease patients22,23. Symptom dissolution (during DBS) and re-emergence (after DBS) typically follow the same timescale. The symptoms that DBS first reduces—e.g., tremor and repetitive movements—are also the first to return22,23. Likewise, the symptoms that DBS corrects last—e.g., locomotion and sustained spasticity—return last22,23. Patterns of recurrence temporally differ because DBS alters different cellular processes, including the desynchronization of neural activity (which occurs instantaneously) and the induction of long-term potentiation (which occurs after the circuit adapts)23,73. In our ataxia model, improvements do not dissipate after 1 week, which indicates that stimulating the cerebellum may remodel motor circuit connectivity73,74 or harness the computational power and plasticity of Purkinje cells. Indeed, Purkinje cell neurotransmission is needed for DBS to be effective (Fig. 6). In addition, one main location at which learning takes place within the cerebellum is at the parallel fiber-and-Purkinje cell synapse, whose activity is modulated by mossy fiber inputs. Mossy fibers relay information from the cortex and spinal cord to the cerebellum in order to refine behaviors. Simple spikes are an output measure of Purkinje cells that represent the action potentials generated by mossy fiber inputs. Here, we found that simple spike properties change in Car8well mice as motor function deteriorates (Fig. 6). When ataxia peaks in Car8well mice, Purkinje cells fire erratically on the whole due to increased pausing. Curiously, the high regularity we find in local Purkinje cell firing (CV2) disappears. Because local variation in simple spike firing is important for motor timing59 and learning75, these findings suggest that the regularity of Purkinje cell firing prior to stimulation may be important for predicting and sustaining responses. Although, we cannot discount the role that age and other circuit firing features play in DBS reception (Fig. 5). Fully elucidating the Purkinje cell properties that impact DBS efficacy (in the presence and absence of age-related changes) will be critical for further optimizing the technique.

No treatment modality to-date fully rescues ataxia, but the greatest benefits have resulted from improving cerebellar and muscle functions. Fryer et al. showed that combining exercise with a genetic rescue reduced ataxia in SCA1 mice69. In humans, pairing brain-computer interfaces with functional electrical stimulation of the muscle recovered voluntary motor control77. Here, we expanded on these results by showing that DBS similarly improve with 13 and 20 Hz DBS (Supplementary Fig. 14), but not with higher frequencies (Supplementary Fig. 1).
combined with motor training yields long-lasting improvements in ataxia. However, in order for DBS to be effective, certain deficits must exist prior to stimulation. This may include prolonged muscle contractions and highly regular trains of Purkinje co-contractions, tend to occur as consistent features. Pre- for different forms of dystonia, a heterogeneous disorder in which specific abnormalities, such as widened EMG signals and muscle co-contractions, tend to occur as consistent features. Preliminary studies in humans and mice indeed support the utility of cerebellar stimulation for dystonia. How effective therapeu- tic cerebellar DBS would be for neurodegenerative ataxias depends on which circuit properties change and how they might mediate the positive effects of stimulation because Purkinje cell loss alone does not block cerebellar DBS effectiveness. Consistent with these data, cerebellar DBS in the shaker rat model of degenerative ataxia shows benefits. In such degenerative mod- els, Purkinje cell degeneration and eventual cell loss induces a plethora of circuit rearrangements, compensatory fiber growth, and death of surrounding cell types. Also, because Purkinje cell degeneration is often accompanied by a reduction in residual cell function in surviving cells. Together, this may create a neural environ- ment conducive to propagating the effects of stimulation. In such a scenario, it is also interesting to consider that DBS is effective when presented with multiple dysfunctional cell types and microcircuits upon which to act. Interestingly, although Car8wdl mice do not have overt neurodegeneration, they do have altered cerebellar patterning. While the distribution of pathological signatures could be more fully appreciated with additional Car8wdl experiments and increasing the number of animals used here, our data strongly support a neurostimulation approach with high therapeutic potential. Our data additionally highlight the dupli- city of cerebellar DBS effects: DBS corrects Car8wdl behav- ioral deficits to support ongoing motor improvements during treatment, but it may also prompt learning mechanisms to sustain those improvements after the treatment regimen has been completed.

Methods

Animals. All animal studies were carried out under an approved IACUC animal protocol according to the institutional guidelines at Baylor College of Medicine (BCM). All of the animals used in this study were maintained in our animal colony at BCM. The Car8wdl mice (Stock #004625) and the C57BLK/SJ control back- ground strain were purchased from The Jackson Laboratory (Bar Harbor, ME). The L7 Cre, Vglut1lox/lox and the Vglut3lox/lox control littermates were genetically engi- neered, as previously described. Note that in the literature, L7 is also referred to as P27 (Purkinje cell protein 2) and Vgat is referred to as Slt32AL (solute carrier family member 3). We bred the control and mutant mice using timed preg- nancies, and we designated noon on the day a vaginal plug was detected as embryonic day (E) 0.5 and the day of birth as postnatal day (P) 0. We used a standard PCR genotyping protocol to differentiate the mutants from the controls, using the primer sequences listed in Supplementary Data 219,80,81. Mice of both sexes and aged P30, P60–P120, and ≥P150 were studied. Biological sex did not impact the mouse behaviors that were studied (Supplementary Fig. 17) after sur- gery, nor did we observe significant behavioral differences between P60, and P120 mice and their littermates implanted with EMG (Supplementary Fig. 17). However, the motor behaviors that were characteristic of the P30 and ≥P150 mutant mice were significantly different from that of P60–P120 Car8wdl mice; therefore, three cohorts (P30, P60–P120, ≥P150) were generated and used in our studies (Supplementary Fig. 17). All of the mice had food and water ad libitum.

Surgical procedures. For all surgical techniques used in these studies, the mice were given preemptive anesthetics (buprenorphine slow release, 1.0 mg/kg s.c., and meloxicam, 5.0 mg/kg SC) with continued applications pro- vided as part of the post-operative procedures. Anesthesia was induced with 3% isoflurane gas and maintained during surgery at 2% isoflurane gas. All surgeries were performed on a stereotactic platform (David Kopf Instruments, Tujunga, CA, USA) with sterile surgery techniques applied throughout the procedures. Imme- diately following surgery, all of the mice were placed in a warming chamber. Animals that underwent surgery for deep brain stimulation (z electrophysiology) were allowed to recover for at least 3 days before any additional experimental analyses were performed. Mice used for WGA conjugated to Alexa 555 neuroa- natomical tracing were perfused 24 h after the surgery.

Deep brain stimulation. Control and mutant mice underwent surgery for DBS and EMG. Twisted bipolar tungsten electrodes with a width of 0.127 mm and a length of 3.5 mm were purchased from PlasticsOne for DBs. Two electrodes were spaced 2.6 mm apart to bilaterally target the interposed cerebellar nuclei and fixed together using Bondic, a UV-light activated bonding agent (Amazon). Next, P30, P60–P120, and ≥P150 mice (n ≥ 3 of each genotype) were deeply anesthetized with isoflurane so that the prepared DBS electrodes could be inserted into the cerebellum using the following stereotactic coordinates, as calculated from Paxinos and Franklin (2001); −6.4 mm (anterior–posterior), ±1.3 mm (lateral–medial), and −2.5 mm (dorsal–ventral).82 These coordinates were used to guide surgery at all of the ages studied because the size of the cerebellar nuclei remains unchanged from P23 to P300.83 The DBS electrodes were secured to the head with C&R Metabond (Parkell, Inc., Edgewood, NY, USA; SKU: #380) and Teets ‘Cold Cure’ Dental Cement (A-M Systems, LLC, Carlsborg, WA, USA, Catalog #525000 and #526000). Mice were allowed to recover in their home cages for at least 3 days before starting the behavioral tests.

Wheat-germ agglutinin (WGA)-Alexa 555 neuroanatomical tracing. To prepare the tracer and equipment, a Drummond capillary tube (7° 3#-000-203-G/XL) was pulled using a micropipette puller (Narishige International Inc., East Meadow, NY), and backfilled with mineral oil84. Approximately 1 μl of 2% WGA-Alexa 555 (Thermo Fisher Scientific, Waltham, MA) diluted in PBS was taken up into the tip of the pulled pipette. Control and Car8wdl mutant mice (n = 3 per genotype) were then anesthetized and prepared for surgery as described above (see the section “Surgical procedures”). A unilateral craniotomy was made above the cerebellar vermis using stereotactic coordinates (anterior–posterior: −6.24 mm, medial–lateral: +1.5 mm from Bregma) obtained from Paxinos and Franklin.82 At a depth of −1.95 mm from the surface of the brain, 4 injections of 18.4 nl WGA-Alexa 555, each spaced 3 min apart, were administered. We used a bulk loading approach for neural tracing with WGA-Alexa 555 because of its reliability as an anterograde tracer when relatively large volumes are loaded. With these volumes, a substantial number of axons and terminals are robustly labeled and upon cutting the tissue, the signal is easily visualized due to the brightness of the signal. Note, however, that bulk loading is typically necessary since the uptake and transport of the tracer in individual cells cannot be controlled. The craniotomy was closed with 10-0 sutures and the skin was sealed with antibiotic ointment (Vaseline). Animals that underwent surgery for deep brain stimulation (z electrophysiology) were allowed to recover for at least 3 days before any additional experimental analyses were performed. Mice used for WGA conjugated to Alexa 555 neuroanatomical tracing were perfused 24 h after the surgery.

In vivo electrophysiology. P30 and ≥P150 Car8wdl and control mice (n ≥ 3) were anesthetized with an intraperitoneal injection of a Ketamine (75 mg/kg)/Dexme- detomidine (0.5 mg/kg) cocktail. Anesthesia was maintained with 0.25% isoflurane. Anesthetized mice were placed into a stereotactic frame and a craniotomy (~2 mm in diameter) was performed over the cerebellum. The craniotomy was drilled −6.4 mm from Bregma and 0 to +2 mm from the midline.82. The electrodes were lowered from 0 to 2 mm, relative to the brain surface, to record Purkinje cell activity. Single-unit, extracellular recordings were then performed, using 5–8 MΩ tungsten electrodes and a motorized micromanipulator (MP-225; Sutter Instru- ment; also see the section “In vivo electrophysiology recordings and data analyses” under “Behavioral analyses”).

Behavioral analyses

DBS and the accelerating rotarod. To better understand the effects of DBS on animal behavior, DBS was paired with the accelerating rotarod to measure motor coordination and motor learning, with EMG to quantify muscle bursting, with the open field assay to track movement, and with footprinting and DigiGait analyses to assess posture. For the accelerating rotarod, there were three assessment periods: “Before ±DBS,” “With or Without DBS,” and “After ±DBS” following the surgical implantation of the DBS electrodes into the cerebellum. During each of these
assessment periods, the mice with surgically implanted DBS electrodes were attached to a system consisting of a Master8 pulse generator and an Iso-Flex stimulator. We found that TA activity was consistent between shorter and longer bouts of locomotion. The number of bursts over time and the mean burst length in the TA muscle were averaged among the animals and compared between-groups using an unpaired, two-tailed Student’s t-test (* p < 0.05). A two-way repeated-measures ANOVA (p < 0.05) followed by a Sidak’s multiple comparisons test was used to determine differences in DBS significance across the TA muscle during the same time frame. The three-way repeated-measures ANOVA (p < 0.05) followed by a Sidak’s multiple comparisons test was used to determine if 13 Hz significantly altered Car8wdl muscle properties relative to control mice, over the 3-week period. The analysis repeated ANOVA with mixed effects followed by a Sidak’s multiple comparisons test was used to determine if 13 Hz significantly altered Car8wdl muscle properties relative to control mice, after 3 days.

**DBS and DigiGait analysis, footprint analysis, and the open field assay.** The open field assay, DigiGait, and footprint analysis revealed the effects of DBS on the general locomotion and gait of P30 and P60–P120 control and mutant mice. The Open Field locomotion system (Omnitech Electronics, Inc., Columbus, OH, USA) used to carry out the open field assay across a 4-week time period. During the first week, mice were acclimated to the Open Field room for 1.5 h, then placed in the Open Field apparatus, where their movement was recorded for 30 min. The movement time and number of locomotor episodes of each mouse were specifically analyzed due to our DBS targeting choice (Accucan Fusion Software, Version 4.7). Because the interposed nucleus controls ongoing motor function, we postulated that its stimulation would alter the time or continuity of movement. White noise was provided in the background during the acclimation and the recording periods. The videos of this interval were collected for each animal and the video was analyzed with the tracking software (Accucan Fusion Software, Version 4.7) following the alternating movement of the left and right hindlimbs.

For DigiGait analysis, one 3-s video was recorded (DigiGait Imager Software (Version 16)), Mouse Specifics, Inc., Boston, MA, USA) for each mouse at each time point. The strain, age, and sex were used to compare same-group differences in activity levels before, during, and after surgery. Unpaired, two-tailed Student’s t-test (* p < 0.05) was used to compare between-group differences in P30 mice. A three-way repeated-measures ANOVA (p < 0.05) followed by a Dunnett’s multiple comparisons test was used to compare between-group differences in activity levels of P30 control and mutant mice. A Dunnett’s multiple comparisons test was performed on these mice. Three-to-five days later, the mice that were previously tracked and operated were reintroduced to the open field assay (Before ±DBS, “Day 6”). Locomotor data corresponding to two additional time points (Before ±DBS and “Day 13”) and 14 days (“Day 20”) after the before ±DBS period, respectively. For each of these additional time points, mice went through the open field apparatus and the recordings followed a consistent protocol. The same two-tailed Student’s t-test (* p < 0.05) was used to compare between-group differences in P30 mice. A three-way repeated-measures ANOVA (p < 0.05) followed by a Dunnett’s multiple comparisons test was used to compare same-group differences in activity levels before, during, and after surgery. Unpaired, two-tailed Student’s t-test (* p < 0.05) was used to compare between-group differences in P30 mice. A three-way repeated-measures ANOVA (p < 0.05) followed by a Sidak’s multiple comparisons test was used to compare between-group differences in activity levels of P30 control and mutant mice. The videos were analyzed with the tracking software (Accucan Fusion Software, Version 4.7) following the alternating movement of the left and right hindlimbs.

For DigiGait analysis, one 3-s video was recorded (DigiGait Imager Software (Version 16)), Mouse Specifics, Inc., Boston, MA, USA) for each mouse at each time point. The strain, age, and sex were used to compare same-group differences in activity levels before, during, and after surgery. Unpaired, two-tailed Student’s t-test (* p < 0.05) was used to compare between-group differences in P30 mice. A three-way repeated-measures ANOVA (p < 0.05) followed by a Dunnett’s multiple comparisons test was used to compare between-group differences in activity levels of P30 control and mutant mice. The videos were analyzed with the tracking software (Accucan Fusion Software, Version 4.7) following the alternating movement of the left and right hindlimbs.

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paw re-contacts the ground to start a new step cycle ("Frame #4"). These numbers were recorded for each individual paw over the span of 16 consecutive steps. The time it took for the hind limb paws to go from Frame #2 to Frame #3 represented the duration of the "braking" period (duration (s) = frame-time conversion factor × ((Frame #2 – Frame #1) + 1)). Likewise, the time it took for the hind limb paws to go from Frame #2 to Frame #5 represented the duration of the "propulsion" period (duration (s) = frame-time conversion factor × ((Frame #3 – Frame #2) + 1)). The "swing" period was then equal to the time it took for the hind xylene twice. Similar calculations were performed to get the total number of frames for the Frame #3 to Frame #4 duration (s) = frame-time conversion factor × ((Frame #4 – Frame #3) + 1)). The "Frame-Time Conversion Factor" was determined by a "Frame-To-Time" conversion calculator in ZapStudio (https://www.zapstudio.com/). This calculator recorded at a rate of 157 frames per second with whole-tissue. The average braking duration of the hind limbs of n = 3 mice. A two- or three-way ANOVA (p < 0.05) with or without mixed-effects was used to determine if differences in forced gait were significantly changed within the same animal (post hoc analysis: Sidak’s multiple comparisons test) and between-genotypes (post hoc analysis: Dunnett’s multiple comparisons test) over time. An unpaired, two-tailed Student’s t-test (p < 0.05) was used to determine if the braking, propulsion, or the swing phases of locomotion in Carb mutant mice differed from that of the control mice.

In vivo electrophysiology recordings and data analyses. Single-unit recordings were attained from at least 3 mice of each genotype with 5–8 MΩ tungsten electrodes (Thomas Recording, Germany) and digitized into Spike2 software (CED, England, Version 7.09). Purkinje cell traces of ≥100 s were analyzed with (Spike2 Version 7.20), MS Excel (Version 16.29.1, 19901700), and MATLAB (Version R2018b). We examined the simple spike properties of F30 and ≥P150 Purkinje cells over a pre-defined period of recordings, including the pause percent, coefficient of variation (CV), and the coefficient of variation of adjacent interspike intervals (ISIs; CV2/ISI).

While the pause (ISI > 5 × mean ISI) percent measures how often spiking within a Purkinje cell is interrupted, the CV measures the variability in spiking (CV = standard deviation of ISIs/mean ISI of a given Purkinje cell). A high CV value indicates irregular firing. Irregular firing is defined by inconsistent intervals of time (ISI = seconds) between spikes. The CV2 measures the local firing regularity by calculating the variability of firing within a short period of two ISIs (CV2 = 2(ISI1 + ISI2)/(ISI1 + ISI2)). The pause percent, CV, and CV2 were all reported as the mean ± standard error of the mean (SEM). Each data point represented an average from the same individual cell. Statistical analyses were performed using unpaired, two-tailed Student’s t-tests (p < 0.05).

Perfusion, basic histology, and tissue staining procedures. To check electrode targeting, survey for cerebellar damage, and analyze muscle histology, mice were anesthetized with 2,2,2-trimethoxyethanol (Avertin) and transected peripherally first with 0.1 M PBS (pH 7.2) then with 4% paraformaldehyde (PFA) after surgery and brain tissue designated for nuclear staining was mounted onto slides and then dried. DAPI-stained tissues were then imaged as percentages from each tissue section from three different animals, per genotype. Muscle fibers were considered "mixed" if the myofibril contained both slow and fast-twitch-related proteins. If a myofibril was not immuno-reactive to the fast or the slow-twitch antibodies, then the myofibril was considered "unstained." The sections were then stained using fluorochrome-conjugated secondary antibodies (i.e., Alexa Fluor 488-, 555-, and 647-immunoglobins) as stated in the Methods section.

Image acquisition and quantification. A Zeiss AxioZoom V16 (Zeiss AxiosVision Software, Release 4.8) was used to image the cerebellum in whole-mount. A Zeiss AxioImager.M2 with Apotome equipped with Z-stack features (Zeiss Zen Pro Software, Version 2.0.0) was used to capture fluorescent images to survey for tissue damage after electrode implantation and stimulation as well as to assess muscle fiber-type composition. The Zeiss AxioImager.M2 (Zeiss Zen Pro Software, Version 2.0.0) was also used for brightfield microscopy. Various histological measurements were quantified from the captured images, including myofibril composition, cerebellar molecular layer thickness, GFAP staining intensity, and Iba1 staining intensity. Muscle fiber-type composition was quantified as percentages from 3 consecutive TA tissue sections from three different animals, per genotype. Muscle fibers were considered “mixed” if the myofibril contained both slow and fast-twitch-related proteins. If a myofibril was not immuno-reactive to the fast or the slow-twitch antibodies, then the myofibril was considered “unstained.” The sections were then stained using fluorochrome-conjugated secondary antibodies.
slow- and fast-twitch muscle fibers in stimulated and non-stimulated control and *Car8*^null^ mice. Calbindin-stained cerebellar tissue from stimulated and non-stimulated P60–P120 control and *Car8*^null^ mice were imaged to measure molecular layer (ML) thickness. We define ML thickness as the distance from the Purkinje cell somata in the Purkinje cell layer (PCL) to the apical surface of the cerebellar cortex. We measured ML thickness in lobule IV/V of 0, 13, and 130 Hz-stimulated mice (n = 3 controls, n = 3 mutants, per condition) to its role in locomotion[^25] and its connectivity to the primary motor cortex[^25] and interposed nucleus[^44]. Six measurements were taken from each animal, then averaged. ML thickness values were plotted in a bar graph, where each point represents the average ML thickness of an individual animal. A two-way ANOVA (p < 0.05) followed by a Sidak's multiple comparisons test was performed to determine whether ML thickness differed between genotypes and stimulation groups. GFAP and Iba1 staining intensities surrounding 6 electrode tracks from three different animals (per genotype, per condition) were quantified, using ImageJ software (Version 1.0). Each image was first duplicated, then the copy was converted into a 16-bit image. The threshold was adjusted on each 16-bit image so that the GFAP- or Iba1-positive areas were accurately highlighted. Using the “Image Calculator” processing function, ImageJ subtracted the established threshold from the original image, yielding a “Mean pixel intensity” value for GFAP or Iba1 staining. Plotted on bar graphs, each point represents the average GFAP or Iba1 staining intensity of an individual animal. A two-way ANOVA (p < 0.05) followed by a Sidak's multiple comparisons test was performed to determine whether the staining intensity of GFAP or Iba1 differed between genotypes and stimulation groups.

Data and statistical summaries. A summary of the data and statistical tests used throughout the study can be found in Supplementary Data 1. The two-tailed, unpaired Student's t-test (p < 0.05) was used whenever one measurement from mutant and control mice were being compared. The one-way ANOVA (p < 0.05) was performed when comparing one measurement from one genotype across different ages. The two-way repeated-measures ANOVA (p < 0.05) was used whenever one measurement from one genotype (*Car8*^null^ or control) was being compared over time and across different stimulation paradigms. The three-way repeated-measures ANOVA (p < 0.05) was used whenever one measurement from both genotypes (*Car8*^null^ and control) was being compared over time and across different stimulation paradigms. In order to limit false positives in our P60–P120 rotardar experiments, one two-way repeated-measures ANOVA was performed, which incorporated all of the possible stimulation paradigms (Supplementary Data 1; No Surgery, 0, 2, 13, 20, 130, unpaired 13, and unpaired 130 Hz). For instance, when tissue was harvested from mice following the last day of stimulation on the rotarod (Day 11) or the EMG signals did not last until Day 3, a separate two-way or three-way ANOVA was conducted with mixed-effects, using the same baseline measurements established “Before” and/or during “sDBS” as the controls. The mixed-effects model uses the maximum likelihood method to estimate missing values. After the ANOVAs were performed, a Sida's, Dunnett's, or Tukey's multiple comparisons test was used to compare the means of the different groups. A Sida's multiple comparisons test was used when comparing a select set of means. A Dunnett's multiple comparisons test was used when comparing experimental means to a control mean. A Tukey's multiple comparisons test was used when comparing all means. Sample sizes were determined based on the statistical criteria for significance in observations rather than an a priori power analysis since the experiments conducted were dependent on the success and/or failure of DBS[^45]. All attempts at replication were successful, unless the DBS electrode was mistargeted. Therefore, no data were excluded from analysis unless the electrodes were mistargeted, as determined through post hoc tissue analyses, or the mice did not complete the rotarod paradigm through Day 11 (Supplementary Fig. 2b). All of the statistical tests were performed by Prism8 software (Version 8.4.3, 471). If significant, the data in Supplementary Data 1 are highlighted in purple.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data and materials used are available in the main text or as supplementary materials. The authors will also provide data and materials upon request. Source data are provided with this paper.
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Author contributions

L.N.M. and R.V.S. conceived and designed experiments. L.N.M., T.L., I.Z., M.E.V.D.H., J.B., J.J.W., and R.V.S. performed experiments. L.N.M., M.E.V.D.H., J.B., and R.V.S. analyzed data. L.N.M. and R.V.S. drafted the manuscript. All authors interpreted results, edited and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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