Neural spike signatures determine the behavioral presentation of cerebellar disease

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Summary
The cerebellum contributes to a diverse array of motor conditions including ataxia, dystonia, and tremor. The neural substrates that encode this diversity are unclear. Here, we tested whether the neural spike activity of cerebellar output neurons predicts the phenotypic presentation of cerebellar pathophysiology. Using *in vivo* recordings as input data, we trained a supervised classifier model to differentiate the spike parameters between mouse models for ataxia, dystonia, and tremor. The classifier model correctly predicted mouse phenotypes based on single neuron signatures. Spike signatures were shared across etiologically distinct but phenotypically similar disease models. Mimicking these pathophysiological spike signatures with optogenetics induced the predicted motor impairments in otherwise healthy mice. These data show that distinct spike signatures promote the behavioral presentation of cerebellar diseases.

Key words
Cerebellum, ataxia, dystonia, tremor, spike activity, optogenetics, classifier model

Introduction

There exists a historical and rich curiosity in understanding the role of the cerebellum in movement, dating back to the pioneering work of Flourens (Flourens, 1841), with an equally long interest in investigating how it alters movement (Holmes, 1917; Turner, 1892). From these earlier studies it was clear that a defective cerebellum causes a range of devastating problems in the ability to control voluntary, intentional actions including coordination, posture, and balance. Accordingly, in humans, the equivalent defects had clear pathophysiological consequences. Patients with various cerebellar lesions showed loss of precise motor coordination, which, as the consequence of disease, is referred to as ataxia, whereas some subjects displayed overt and sometimes exaggerated oscillatory movements, consistent with tremor (Holmes, 1917, 1939; Turner, 1892). In other cases, the examiner would report uncontrolled muscle contractions, which is now a contributing feature of dystonia (Balint et al., 2018). These classic observations underscored the heterogeneity of motor disturbances caused by cerebellar dysfunction. But, even then, the question of how the cerebellum creates such behavioral diversity was already imminent.

Consistent with the outcomes of removing or lesioning the cerebellum in pigeons, dogs, monkeys, and humans (Holmes, 1917, 1939; Turner, 1892), the heterogeneity of cerebellar movement disturbances was later confirmed in more modern models, such as the mouse. Much of the current excitement about the cerebellum spawned from these initial genetic models because of the overt motor disturbances that were caused by spontaneous mutations in genes that are now known to be associated with cerebellar development or cerebellar degeneration (Falconer, 1951; Sidman et al., 1965). Prior to genetic sequencing though, mutant mice such as *hot-foot* (Guastavino et al., 1990), *weaver* (Rakic and Sidman, 1973), *trembler* (Falconer, 1951), *waddles* (Jiao et al., 2005), *staggerer* (Sidman et al., 1962), *stumbler* (Caddy et al., 1981), *tottering* (Green and Sidman, 1962), and *lurcher* (Phillips, 1960) were named according to phenotype of their abnormal movements, which are as diverse as their names imply. Modern techniques and approaches now aim at determining the mechanisms and roles of the cerebellum as they relate to driving different behaviors in these classic models (Pan et al., 2020; Snell et al., 2022). Nevertheless, more than half a century since the first descriptions of cerebellar mutants, a core question remains unanswered: how does cerebellar circuit dysfunction lead to unique motor disturbances?

To begin addressing how cerebellar circuits generate behavioral diversity, we used an intersectional genetics approach to mark, map, and manipulate specific types of synapses in the cerebellum. Our approach silences genetically defined populations of synapses by selectively deleting the genes that encode the vesicular GABA transporter, *Vgat*, or the type 2 vesicular glutamate transporter, *Vglut2*, from genetically targeted populations of cells. Loss of GABAergic neurotransmission from Purkinje cells, which provide the sole output of the cerebellar cortex, caused uncoordinated movements and disequilibrium that were indicative of ataxia (Figure 1A) (White et al., 2014). In contrast, eliminating glutamatergic neurotransmission from climbing fibers, which synapse onto Purkinje cells, caused twisting postures and hyperextended limbs that is consistent with dystonia (Figure 1A) (White and Sillitoe, 2017). In addition, we found that systemic injection of the tremorgenic drug harmaline caused hyperactivation of climbing fibers and rhythmic bursting activity in Purkinje cells. Optogenetically modulating Purkinje cell to cerebellar nuclei projections in a bursting pattern induced oscillatory tremor movements (Figure 1A) (Brown et al., 2020a). Together, these studies established that different manipulations of Purkinje cell inputs or outputs, and consequently Purkinje cell and nuclei neuron spike signals, can cause diverse behavioral deficits (Brown et al., 2020a; White and Sillitoe, 2017). Given the heterogeneity and even comorbidity of these behaviors in a single disease model (White et al., 2016a), the main question that arises is, do these neural signals represent unique pathophysiological signatures that can drive the predominant behavioral defects used to characterize different motor diseases?

Results

Spike signatures are different between archetypical ataxia, dystonia, and tremor models

We first compared the spike activity between mouse models for ataxia (*L7^{Cre};Vgat$^{fl/fl}$*, (Brown et al., 2020a; White et al., 2014)), dystonia (*Ptf1a$^{Cre/+}$;Vglut2$^{fl/fl}$*, (Brown et al., 2022; White and Sillitoe, 2017)), and tremor (harmaline injection, (Brown et al., 2020a; Handforth, 2012)) (Figure 1A). We chose these three mouse models because of the reliability, severity, and high penetrance of the ataxic, dystonic, and tremor symptoms within each respective group. We performed *in vivo* electrophysiology recordings of the spike activity in cerebellar nuclei neurons of the interposed nucleus (Figure 1B,C), and described the spike firing features using twelve parameters (see Methods). We focused our recordings in the interposed nucleus based on the hypothesis that the cerebellum communicates ongoing motor signals to other brain regions through this nucleus (Low et al., 2018) and deep brain stimulation (DBS) of this region successfully reduces motor impairments in mouse models for ataxia (Miterko et al., 2021), dystonia (White and Sillitoe, 2017), and tremor (Brown et al., 2020a). We then trained a supervised classifier model to find which of the parameters best distinguished the spike characteristics of neurons recorded in each mouse model (Figure 1D). The classifier model identified the coefficient of variance (CV) of interspike intervals (ISI) as the best differentiator of neurons recorded in ataxic and control mice (lower CV) from neurons recorded in dystonic and tremoring mice (higher CV). CV is a measure of variability that increases due to pauses between spikes or fluctuations in the firing rate (Figure 1B) and is unusually elevated in our dystonic and tremor mouse models (Figure 1B”). Next, the classifier model identified CV2 (Holt et al., 1996) as the best differentiator of neurons recorded in ataxic mice (lower CV2) from control mice (higher CV2). CV2 measures the irregularity of interspike intervals with less influence from the overall firing rate. It is extraordinarily low in the highly regular spike activity observed in our ataxia model (Figure 1B’’). Last, the classifier model identified that the median ISI^{-1}, which measures the median instantaneous firing rate, as the best differentiator of neurons from the dystonic mice (lower median ISI^{-1}) compared to those recorded in mice with tremor (higher median ISI^{-1}) (Figure 1C).

We then validated whether this classifier model could reliably predict the behavioral phenotypes of mice based solely on the spike activity of single cerebellar nuclei neurons. We found that the classifier model indeed predicted the different predominant phenotypes in each mouse model correctly for the majority of neurons in the validation data set (control: 80% of neurons recorded in a control mouse had a control signature; ataxia: 78%; dystonia: 65%; tremor: 56%) (Figure 1E). Likewise, the converse analysis showed that most neurons of a specific signature were recorded from the mouse model with that phenotype (control: 59% of neurons with a control signature were recorded in a control mouse; ataxia: 89%; dystonia: 65%; tremor: 73%) (Figure 1E-G). These data support the hypothesis that spike signatures can predict the behavioral phenotype of mouse models with different motor symptoms.

Spike signatures are shared across mouse models with similar behavioral phenotypes

Next, we set out to test whether the spike signatures identified by the supervised classifier learner model were shared across mouse models that exhibit similar abnormal behavioral phenotypes, but are associated with different etiologies. To resolve this problem, we started by ensuring that the classifier learner would not inadvertently predict phenotypes in mice that did not exhibit clear ataxic, dystonic, or tremor phenotypes. To this end, we used spike recordings from control animals from different experiments (Figure 2A), and mice with ataxia, dystonia, and tremor phenotypes that were less severe than those of our select models (Figure 1). This included mice that have an onset of ataxia that occurs early in the disease’s progression (4 month old *Sca1$^{154Q/+}$* mice (Coffin et al., 2023), Figure 2B), mild dystonia (*Pdx1^{Cre};Vglut2$^{fl/fl}$* mice ((Lackey, 2021), Figure 2C), and mild tremor (*Thap1$^{+/-}$* mice (van der Heijden et al., 2020), Figure 2D). In none of these models did we observe that the majority of recorded neurons could collectively predict a disease phenotype, indicating that our classifier model is a sensitive predictor of behavioral deficits in mouse models that express distinct features that designate them as ataxia, dystonia, and tremor (Figure 2I, J).

Next, we set out to test whether our classifier model correctly predicts phenotypes in mice with severe ataxic, dystonic, and tremor behaviors. For this analysis, we included mice with ataxia onset that

occurs late in disease progression (10-12 month old *L7^{Cre};Ank1$^{fl/fl}$* mice (Stevens et al., 2021), Figure 2E) and a drug-induced model of severe dystonia (ouabain infusion on the cerebellum (Calderon et al., 2011; Fremont et al., 2014), Figure 2F). We also analyzed the spike activity in mice with a compound motor phenotype that includes comorbidity of ataxia, dystonia, and tremor (*Car8$^{wdl/wdl}$* (Jiao et al., 2005; Miterko et al., 2021; White et al., 2016a; Zhou et al., 2022), Figure 2G). In these mice, the tremor can be treated with propranolol ((Zhou et al., 2022), Figure 2H). In all these models, fewer than half of the neurons exhibited the control signature, yet the neurons in the severe ataxia model exhibited the dystonia signature (Figure 2I-J). The majority of neurons in the severe dystonia model had the dystonia signature (Figure 2I-J). Interestingly, the majority of neurons in the *Car8$^{wdl/wdl}$* mice, which have a pronounced tremor, showed a tremor signature. This tremor signature changed into a mixed dystonia and ataxia signature upon treatment with the tremor-reducing drug propranolol (Figure 2I-J). Taken together, we found that the dystonia and tremor signatures are indeed shared across mouse models with severe dystonia and tremor. Intriguingly though, mice with severe ataxia can exhibit neuronal firing activity that would be characterized as dystonia-like, and in fact, if a mutant does contain a relatively large proportion of dystonia-like cells, these neural defects could contribute to the overall phenotype by influencing the predominant behaviors that the mice express.

Different spike signatures can be generated by the same canonical cerebellar circuit

Next, we set out to investigate whether the different spike signatures could be generated by the same canonical cerebellar circuit. We reasoned that the observed spiking signatures were not dependent on developmental changes in circuit connectivity (*L7^{Cre};Vgat$^{fl/fl}$*; *Ptf1a$^{Cre/+}$;Vglut2$^{fl/fl}$*; *Pdx1^{Cre};Vglut2$^{fl/fl}$*), neurodegeneration (*Atxn1$^{154Q/+}$; L7^{Cre};Ank1$^{fl/fl}$*), or the effects of transgene expression (*Atxn1$^{154Q/+}$*; *Car8$^{wdl/wdl}$*), gene deletion (*Thap1$^{+/}$*), or drug targeting (harmaline, ouabain, propranolol) in brain regions outside of the cerebellum. We expressed a light-sensitive opsin, channelrhodopsin (ChR2), in Purkinje cells. Upon light activation, Purkinje cell firing rate increases (Supplemental Figure 1A-B) and nuclei neurons are inhibited via the GABAergic neurotransmission from Purkinje cells (Figure 3A-C). We leveraged this circuit connectivity and optogenetic strategy to induce distinct spike signatures in cerebellar neurons. We chose three stimulation paradigms. The ataxia stimulation was a continuous 50 Hz square pulse stimulation (mimicking the lack of modulation by Purkinje cells in ataxia models); the dystonia stimulation was a 50 Hz square pulse stimulation that was on for at least 500 ms with a 75% chance of a 250 ms pause between periods of stimulation (to mimic slow and irregular dystonia signature); and the tremor stimulation was a 10 Hz sinusoidal stimulation (to mimic the fast, irregular, and rhythmic tremor signature). We performed *in vivo* recordings in awake, head-fixed mice, and confirmed that different optogenetic stimulation paradigms caused distinct changes in Purkinje cell spiking activity (Supplemental Figure 1). The different stimulation paradigms also caused distinct spike signatures in cerebellar nuclei neurons (Figure 3D). We found that the ataxia stimulation paradigm induced a variety of spike signatures that were predicted to be ataxia (2/9), dystonia (2/9), or tremor (2/9), which is in line with our findings that the ataxia signature is more variable across ataxia mouse models (Figure 2). Importantly, we found that fewer than half of the cells recorded with the ataxia stimulation exhibited a control signature (Figure 3E). Additionally, we found that the dystonia and tremor stimulation reliably induced spike signatures that our classifier model defined as dystonic (7/7 cells) or as tremor signatures (9/9 cells), respectively (Figure 3E). Taken together, we found that ataxic, dystonic, and tremor signatures can be generated by the same, otherwise healthy, cerebellar circuit. These data indicate that no anatomical changes are required to induce disease-associated spike signatures in the cerebellum.

Spike signatures can induce distinct motor phenotypes that mimic disease-related behaviors

During the head-fixed stimulation experiments, we observed behavioral responses that reflect a baseline representation of how the different signatures might drive behavior (Video 1). To explore this further in freely moving mice, we investigated whether the different spike signatures directly drive unique motor disturbances. To this end, mice were implanted with optical fibers that were bilaterally targeted to the interposed cerebellar nuclei (Figure 4A-C). This allowed the mice to freely move during stimulation and

all behavioral assays (Figure 4D). Changes in behavior were immediate and severe, as soon as the stimulation began. Each stimulation paradigm resulted in a different constellation of behaviors with the ataxia stimulation paradigm resulting in imprecise movements, the dystonia stimulation paradigm resulting in prominent dystonic posturing, and the tremor stimulation paradigm resulting in severe tremor (Video 2). Therefore, we assessed the mice for changes in gait, presence of dystonic movements, and severity of tremor. No single measurement can perfectly encapsulate the complexity of ataxia, dystonia, and tremor – all of which can appear in combination with the others (Hagerman and Hagerman, 2015; Nibbeling et al., 2017; Pandey and Sarma, 2016). Additionally, each phenotype may affect the measurement of others. This was particularly apparent with measurements of gait. We found that all stimulation paradigms affected gait, resulting in visibly different foot placement (Figure 4E-F) and movement down the footprinting corridor (Video 3). Quantitatively, this was evident in shorter steps and less precision of fore and hindpaw placement (Figure 4I-K). We also considered the behavior of the mice in an open, flat, plexiglass arena for signs of dystonic movements (Figure 4L, Video 2). The dystonia stimulation paradigm resulted in the most frequent and strongest dystonic movements, while we also observed dystonic movements with the ataxia paradigm and abnormal movement – namely severe tremor – was recorded with the tremor stimulation paradigm (Figure 4M). Mice were also placed in a custom-made tremor monitor (Brown et al., 2020a) where they could freely ambulate during stimulation while an accelerometer mounted under the arena detected changes in acceleration (Figure 4D). While the ataxia stimulation paradigm did not significantly increase tremor from baseline, both the dystonia and tremor stimulation paradigms resulted in a significantly increased tremor at 10 Hz (Figure 4N-P). Though dystonia is often observed with tremor, it is possible that the detection of tremor in these animals was due to the jerkiness of their dystonic movements rather than an increase in smooth, rhythmic tremor that is more often associated with tremor disorders. Indeed, the tremor paradigm resulted in the most severe tremor of all conditions, producing a tremor severity that was more than ten-fold greater than that of the dystonia stimulation paradigm. Together, these measurements produced a complex representation of the underlying phenotypes. All three stimulation parameters resulted in a behavioral repertoire that was significantly different from baseline and, while there was some overlap of features, each stimulation paradigm produced the distinct and predicted motor phenotype for each respective stimulation paradigm (Figure 4Q-T).

Discussion

In this study, we tested whether distinct spiking signatures in the interposed cerebellar nuclei explain how cerebellar dysfunction can cause distinct motor impairments and movement disorders. We found that the cerebellum can generate a range of dysfunctional spiking patterns by comparing spike activity across multiple mouse models of cerebellar disease. We found disease-specific spike signatures using a classifier learner model, which allowed us to discover specific parameters and their corresponding cutoff values that could distinguish the spike activity associated with these different disease states. While a myriad of spiking activity can cause ataxia, specific spiking signatures are strongly associated with dystonia and tremor. Using these signatures, we then tested whether we could optogenetically induce them in an otherwise normal circuit. We found that, indeed, the same neurons can generate healthy spike activity as well as a spectrum of disease-like spike activities. This suggests that it is possible for neural signals to shift back and forth between healthy and disease states, potentially resulting in the transientness of behavioral impairments in certain cerebellar disorders. We then tested whether these optogenetically-induced disease-associated spike signatures could elicit similar behavioral abnormalities in absence of any other primary genetic or circuit defects in awake and freely moving mice. The predominant behaviors that characterize each disease condition were successfully recapitulated. These data provide compelling evidence for the reliance of neurological phenotype presentation on the presence of circuit misfiring. Moreover, despite the dramatically different behavioral outcomes, the potential overlap and shared spike features, such as different types of “bursts”, raise the strong possibility that co-expression or comorbidity of different motor disease behaviors may arise due to a spectrum of spike signal defects. Our work suggests that the most successful therapeutic avenues may maintain cerebellar signals within the healthy range without inadvertently inducing a different pathophysiological signature.

There has been (Slaughter et al., 1970), and there still remains (Diniz et al., 2021), a great interest in understanding how altered cerebellar signals influence human behavior and disease. A pressing need to better define the architecture of these spiking abnomalities has arisen because of the success in using deep brain stimulation (DBS) and the potential for better tuning of the stimulation parameters for greater efficacy in treating different cerebellar-related disorders (ataxia: (Cury et al., 2021; Teixeira et al., 2015); dystonia: (Brown et al., 2020b); tremor: (Horisawa et al., 2021; Paraguay et al., 2021); ataxia, dystonia, tremor: (Diniz et al., 2021)). A parallel motivation has fueled rodent studies to define the anatomical targets, stimulation parameters, and outcome efficiacy in detail (Anderson et al., 2020; Cooperrider et al., 2014; Miterko et al., 2019, 2021; White and Sillitoe, 2017). Although the exact mechanism(s) of DBS remains unclear (Miterko et al., 2019; Schor et al., 2022), there is strong support that at least one target of DBS is the actual neuronal signal itself (which the DBS may modify, enhance, dampen, and perhaps even interfere with the given neural spike defects). Indeed, the ability to alter cerebellar spike activity (Nashold and Slaughter, 1969) has driven the investigation of the signal properties (Slaughter et al., 1970). Evidently, neurotransmission of the faulty activity patterns is required for the expression of disease behaviors (Stratton and Lorden, 1991). Recent works strongly support the hypothesis that changes in activity (rate, pattern, or both) could instigate a range of movement abnormalities (Brown et al., 2020a; Fremont et al., 2014, 2017; LeDoux and Lorden, 2002; White and Sillitoe, 2017). However, the current work defines the individual potential of these altered cerebellar signals to initiate specific motor changes. Dystonia can be reliably associated with burst activity and tremor with highly rhthmic oscillations in the cerebellar nuclei neurons. Ataxia may be caused by a variety of changes that are different from the dystonia and tremor signatures in the severity of misfiring or proportion of neurons affected. Together, these data unveil a critical role for the cerebellum in triggering disease behaviors, with cauasative signals likely originating locally in its circuitry. In this regard, it is intriuiging that even in the population of normal cerebellar nuclei neurons, a minor but noticiable representation of ataxic, dystonic, and tremorgenic neurons is present. Perhaps part of the disease phenotype emerges as an exagerated response of these already phenotypic cells. Each disease may therefore arise as a result of a change in the balance and representaion of neural signatures.

Methods

Animals: Mice were housed in a Level 3, AALAS-certified vivarium. The Institutional Animal Care and Use Committee (IACUC) of Baylor College of Medicine (BCM) reviewed and approved all experimental procedures that involved mice. We used the following transgenic mouse lines: *L7*^{Cre} (also known as *Pcp2*^{Cre}) (Lewis et al., 2004), *Vgat*^{fl} (JAX:012897) (Tong et al., 2008), *Ptf1a*^{Cre} (JAX:023329) (Nakhai et al., 2007), *Vglut2*^{fl} (JAX:012898) (Tong et al., 2007), *Thap1*$^{+/-}$ (Ruiz et al., 2015; van der Heijden et al., 2020), *Pdx1*^{Cre} (JAX:014647) (Hingorani et al., 2003), *Car8*$^{wdl/wdl}$ (JAX:004625) (Jiao et al., 2005), *Sca1*^{154Q} (JAX:005601) (Watase et al., 2002), *Ank1*^{fl} (JAX:036512) (Stevens et al., 2021), and *Ai32* (*Rosa26*$^{lsl\text{-}ChR2\text{-}eYFP}$, JAX:024109) (Madisen et al., 2012). We included mice from both sexes. We used ear punches from pre-weaned pups for PCR genotyping to identify the different transgenic alleles.

Headplate and craniotomy surgery for electrophysiology recordings: Prior to all recordings, we performed a surgery to attach a headplate over bregma and make a craniotomy over the interposed nucleus. This allowed us to collect stable recordings of cerebellar neuron activity while the mouse was awake and with or without a severe motor phenotype. This surgery was detailed in our previous publication (White et al., 2016b). In short, mice were given preemptive analgesics including a subcutaneous injection of buprenorphine and meloxicam. Anesthesia was induced using inhaled isoflurane. During the surgery, the mice were kept on a heating blanket to maintain body temperature. Fur was removed from the surgery site using depilatory cream (Nair) and a small incision in the skin was made over the skull. Next, we used a dental drill to make a ~2 mm diameter craniotomy over the interposed nucleus (6.4 mm posterior and 1.3 mm lateral to Bregma). The craniotomy was surrounded by a round chamber and filled with antibiotic ointment until the day of the recording. The recording chamber was closed with a screw top or silicone cap. We also placed a headplate with a hole over Bregma. A small piece of wire was placed vertically over Bregma to identify this anatomical marker on the day of recording. The recording chamber and headplate were attached to the skull using a combination of C and B Metabond Adhesive Luting Cement (Parkell) and methyl methacrylate dental cement (A-M Systems). Mice were allowed to recover from the surgery for at least three days before we began conducting the *in vivo* electrophysiological recordings.

Standard in vivo electrophysiological recordings in awake mice: We performed *in vivo* electrophysiology recordings according to experimental procedures detailed in our previous publications (Brown et al., 2020a; van der Heijden et al., 2020; White et al., 2016b). In brief, we placed mice on a rotating foam wheel and stabilized their heads by screwing the implanted headplates to the recording rig. We removed the antibiotic ointment from the recording chamber and replaced it with sterile physiological saline solution (unless otherwise specified). We measured the coordinates of Bregma to determine the coordinates where the electrode would penetrate the surface of the cerebellum. We used tungsten electrodes (2-8 MΩ, Thomas Recording) attached to a preamplifier head stage (NPI Electronic instruments) for our recordings. The position of the recording electrode was controlled using a motorized micromanipulator (MP-255; Sutter Instrument Co.). Neural signals were amplified and bandpass filtered (0.3-13 kHz) using an ELC-03XS amplifier (NPI Electronics) before being digitized using a CED board. All signals were recorded using Spike2 software (CED). We only included neurons recorded between 2.5 and 3.5 mm from the surface that did not exhibit complex spikes (characteristic for Purkinje cell firing) in our analyses of cerebellar nuclei neuron firing patterns. This paper includes reanalyzed data from previously reported studies (Brown et al., 2020a; Stevens et al., 2021; van der Heijden et al., 2020; White and Sillitoe, 2017).

Spike sorting and analyses: We previously showed that recording duration influences the estimation of neural firing parameters (van der Heijden et al., 2022a). Therefore, all parameter estimates in this paper are based on 30 seconds (s)-long recordings. We used Spike2 software to sort out spikes from electrophysiological recordings. Information about spike timing were imported and saved in MATLAB databases using custom written code. We described the spike analyses using twelve parameters investigating the properties of interspike intervals (ISI) within the recording; (1) $firing\ rate = \frac{spikes}{s}$; (2)

$mean\ instantaneous\ firing\ rate = mean\ (ISI^{-1})$; (3) $median\ instantaneous\ firing\ rate = median\ (ISI^{-1})$; (4) $CV = \frac{stdev(ISI)}{mean(ISI)}$; (5) $CV2 = mean\left(2 \times \left|\frac{ISI_n - ISI_{n-1}}{ISI_n + ISI_{n-1}}\right|\right)$; (6) $skewness = \frac{median\ instantaneous\ firing\ rate - firing\ rate}{firing\ rate}$; (7) $ISI_{25} = \%\ ISI > 25\ ms$; (8) $ISI_{100} = \%\ ISI > 100\ ms$; (9) $inter\ burst\ pause = mean\ (ISI > (5 * mean\ (ISI))$; (10) $kurtosis = \%\ ISI\ at\ mode(ISI)$; (11) $rhythmicity\ index = \frac{a_1}{z} + \frac{b_1}{z} + \frac{a_2}{z} + \frac{b_2}{z} + \cdots$; (12) $oscillation\ peaks = number\ of\ peaks\ "a"$. For the calculation of 11 and 12, we performed an autocorrelation analysis for all spikes in the 30 s recording, calculated the rhythmicity index, and found oscillation peaks as previously described (Lang et al., 1997; van der Heijden et al., 2021). We used a bin width of 5 ms. The first oscillation peak was determined as the highest bin between 10 ms and 1.5 times the mean ISI for a given neuron. The difference between the baseline level and the height of the peak was denoted as a_1, the difference between baseline and the depth of the trough was denoted as b_1, and z was the difference between baseline and the total number of spikes included in the analyses. Each subsequent peak was determined as the highest bin between the delay-time of the previous trough and a_n+a_1+10 ms, where a_n is the time of the previous peak. The first trough was determined as the lowest bin between the first peak (a_1) and a_n + a_1. Peaks and troughs were only accepted if their sum was higher than four times the standard deviation of autocorrelation between 0.96 and 1 s lag-time, or if the peak was higher than baseline plus two times the standard deviation and the trough was lower than baseline minus two times the standard deviation.

Supervised classifier learner: We trained our classifier learner using cerebellar nuclei neuron recordings from mixed background control mice (control), *L7^{Cre};Vgat$^{fl/fl}$* mice (ataxia), *Ptf1a^{Cre};Vglut2$^{fl/fl}$* mice (dystonia), and harmaline-injected mice (tremor). We have previously tested these models and consider them as reliable archetypical models for cerebellar movement disorders (Brown et al., 2020a; White and Sillitoe, 2017; White et al., 2014). For the analyses, we reanalyzed previous recordings (Brown et al., 2020a; Stevens et al., 2021; van der Heijden et al., 2020; White and Sillitoe, 2017; Zhou et al., 2022) in addition to newly acquired ones. We only included recordings with a duration of 30 s or longer and calculated parameter estimates as described above. If recordings were longer than 60 s, we also calculated parameter estimates for the second 30 s period. We randomly assigned the parameter estimates calculated from the first 30 s of the recording to the training or validation data set and, when available, we assigned the parameter estimates from the second 30 s of the recording to the remaining data set. This resulted in a training set comprised of 29 neurons from 9 control mice, 19 neurons from 5 ataxic mice, 19 neurons from 8 dystonic mice, and 21 neurons from 6 tremoring mice. The validation set contained 30 neurons from 9 control mice, 18 neurons from 5 ataxic mice, 17 neurons from 7 dystonic mice, and 18 neurons from 5 tremoring mice.

We used the built-in supervised machine learning, MATLAB Classification Learner APP (The Mathworks Inc, version R2021a) to define spike signature parameter value cutoffs. We imported the parameters describing the spiking activity from an Excel (Microsoft) worksheet. We used “Group” (control, ataxia, dystonia, or tremor) as the “Response Variable” and the 12 parameters described above as the “Predictor Variables.” We trained a “Coarse Tree” with maximum number of splits = 3, split criterion = Gini’s diversity index, and surrogate decision splits = off. We exported the trained model to the workspace to validate the classifier learner. We assigned signature identity to recordings based on the trained classifier tree.

Optopatcher in vivo electrophysiology recordings in awake mice: Optopatcher experiments were performed as previously described (van der Heijden et al., 2022b). We prepared the mice for recording by performing a headplate and craniotomy surgery as described above. The electrophysiology rig and setup were the same as described above with the following differences: We used an optopatcher device (ALA Scientific Instruments Inc) that allows for the placement of a custom-made optical fiber (Thorlabs, #FT200UMT) within a glass recording electrode (Harvard Apparatus, #30-0057). The tip of the optical fiber was placed near the tip of the recording electrode and was illuminated via a 465 nm LED light source

(ALA Scientific Instruments Inc). On the day of the recording, we pulled glass electrodes (Sutter Instruments, #P-1000), filled the electrodes with physiological saline, and measured their impedance using an ELC-03XS amplifier (NPI Electronics) before recording. Only electrodes with 2-15 MΩ impedance were used. Light stimulation was triggered using custom Spike2 scripts paired with a CED board (CED). All optopatcher recordings were performed in *L7^{Cre};Ai32* mice that express channelrhodopsin in Purkinje cells. Nuclei neurons included in our analyses were cell recordings between 2.5-3.5 mm from the surface of the cerebellum and were inhibited by brief light stimulation at 465 nm. After we found and isolated a cell, we slowly ramped up the brightness of this brief stimulation until we found the minimal light power that modulated the spiking activity. This minimal power was then used to stimulate the cell with the various test parameters during the recording session.

Optogenetic light stimulation paradigms for optopatcher recordings and behavioral assays: We used the following stimulation parameters to measure signature-specific responses; ataxia: 50 Hz (10 ms light on/ 10 ms light off) square pulses; dystonia: at least 500 ms of 50 Hz (10 ms light on/ 10 ms light off) square pulses interspersed with at 75% chance of 250 ms pauses in stimulation; tremor: 100 Hz pulses (5 ms light on/ 5 ms light off) overlaid with a 10 Hz sinusoid (50 ms parabolic increase and decrease of light power followed by 50 ms light off).

Bilateral optic fiber implant surgery for in vivo behavioral assays: We implanted mice with optical fibers (ThorLabs, #FT200UMT) targeted bilaterally to the interposed cerebellar nuclei to assess the motor phenotypes that result from our stimulation parameters. We previously described this surgical procedure (Brown et al., 2020). Briefly, the mice were given subcutaneous injections of sustained-release buprenorphine and meloxicam as preemptive analgesics. Anesthesia was induced with 3% isoflurane gas and the anesthetic plane was maintained with 2% isoflurane gas. The mice were placed on a heating blanket and the head was stabilized in a stereotaxic frame (David Kopf Instruments). Fur was removed from the top of the head using depilatory cream (Nair). The surgical site was cleaned using alternating applications of betadine scrub and alcohol. An incision was made in the skin to expose the skull from anterior to bregma to the posterior aspect of the occipital plate. The fascia was removed from the top of the skull and a scalpel was used to etch fine grooves into the top of the skull. A small craniotomy was made in the parietal plate with a dental drill in order to attach a skull screw (00-90 x 1/16 flat point stainless steel machine screw) to anchor the implant to the skull. The skull screw was advanced only until the point that it was stable in the skull and care was taken to ensure it did not contact the brain. The implant sites were determined by measuring 6.4 mm posterior and ±1.5 mm lateral to bregma. Small craniotomies were made at these sites using a dental drill. The base of the fibers were placed on the surface of the cerebellum and were advanced ventrally for 2 mm. A small amount of antibiotic ointment was placed around each fiber to prevent Metabond or dental cement from entering the craniotomy. Metabond was applied over the entire exposed area of skull and around the skull screw and fibers. Two short segments of wire were embedded in the Metabond to allow the experimenter to hold the mouse's head while attaching and removing the fiber patch cables from the implant. Dental cement was placed over the Metabond. The mice were allowed to recover for at least 3 days before any behavioral assessments were made. Subcutaneous injections of meloxicam were provided every 24 h during the recovery period. Eight mice (number of mice, N) were implanted and tested with all three behavioral assays.

Gait analysis: Measurements of gait were made for all mice both before (baseline) and during each stimulation parameter. To do this, the mice were briefly scruffed and then contrasting colored non-toxic paints were applied to the soles of their forepaws and hindpaws (blue paint for forepaws and red paint for hindpaws). The mice were then gently set down on a piece of blank white paper positioned between two parallel plexiglass barriers with a dark enclosed area at the end of the corridor. Adult mice are naturally inclined to walk towards the dark enclosed area, meanwhile depositing paint on the paper with each footstep. The stimulation, if present, was extinguished once the mice entered the enclosed area. The mice were allowed to rest for at least 2 min between each trial. A trial was considered successful if

there were at least four hind and forepaw prints for both the left and right feet that were visible, steady (the mouse was neither running or stopping midway through the series of footsteps), and in as straight of a line as possible (the mouse was not actively turning during the trial). Optic patch cables were connected to the implant during every trial (including baseline runs when stimulation was not present). At least two successful trails were collected per stimulation parameter. For analysis, measurements from two trials of the same type were averaged to determine the values for each mouse. These measurements were stride, the distance from one footprint to the next from the same foot and hind to fore distance, and hind-to-fore distance, the distance from one hindpaw placement from a corresponding forepaw placement on the same side of the body. Three of each measurement were made per footprint and were averaged to determine the measurements per foot, per trial. These trials were then averaged to determine the final measurements for each mouse. A repeated-measures one-way ANOVA with a Tukey multiple comparison adjustment was performed to determine significance. Significance values were indicated as not significant (ns) if $p > 0.05$, * = $p \leq 0.05$. ** = $p \leq 0.01$.

Dystonia rating scale: Mice were placed in a rectangular plexiglass arena in order to phenotypically rate the frequency and severity of dystonic behavior as described previously (Jinnah et al., 2000; Pizoli et al., 2002). The videos were captured to include the animals' behavior before (baseline) and during each stimulation paradigm (ataxia, dystonia, and tremor). Each stimulation period lasted 2 min, during which the mouse was allowed to ambulate freely as well as in response to disturbance by the experimenter. The rating period excluded the first 10 s after stimulation was initiated to avoid including behavior in response to the sudden application of stimulation. Mice were given a score of 0 if no motor abnormalities were identified, 1 if there was abnormal motor behavior that was not obviously dystonic (such as severe tremor), 2 if there was a mild motor phenotype that included dystonic behaviors that may occur only in response to being disturbed by the experimenter or unprovoked (such as brief hyperextension of the limbs or extension of the neck and/or back), 3 if there was moderate severity or frequent unprovoked dystonic behaviors, 4 if there were severe unprovoked dystonic behaviors, and 5 if there were severe unprovoked dystonic behaviors that made goal-directed ambulation extremely difficult or impossible for an extended time. A detailed description of dystonic phenotypes in mice can be found in Brown et al. 2022 (Brown et al., 2022). Wilcoxon matched-pairs signed rank tests were performed with a post-hoc Holm-Sidak method for p-value adjustment in order to determine significance. Significance values were indicated as not significant (ns) if $p > 0.05$, * = $p \leq 0.05$. ** = $p \leq 0.01$.

Tremor analysis: The amplitude and frequency of tremor before (baseline) and during optogenetic stimulation was measured using a custom-made tremor monitor as previously described (Brown et al., 2020; van der Heijden et al., 2021; Zhou et al., 2022). The mice were implanted with optical fibers targeted to the interposed nucleus as described above. LED drivers were connected to the implant via optical patch cables and placed above the tremor monitor chamber. The tremor monitor chamber is a clear plexiglass box that is suspended by elastic cords that are connected to each corner of the box. An accelerometer is securely mounted to the bottom of the box to detect the tremor of the mouse within the box. The mice were placed in the chamber and were able to freely ambulate while in the box. They were given 2 min to acclimate to the novel tremor monitor environment before 2 min duration baseline recordings were made. The mice were then stimulated with the ataxia, dystonia, and tremor stimulation parameters for 2 min per stimulation period. At least 2 min were allowed to elapse between stimulation periods. Mice were encouraged to ambulate a similar amount during all recording periods. The output from the tremor monitor was amplified and lowpass filtered at 5 kHz (Brownlee Precision, Model 410) before being digitized by a CED board and analyzed using Spike2 scripts. Tremor monitor recordings were centered on zero and downsampled using the Spike2 interpolate function. A power spectrum analysis with a Hanning window was performed on each recording period. The peak power was calculated as the maximum power between 0 and 30 Hz, which is consistent with the range that we typically observe for physiological and pathological tremor in mice (Brown et al., 2020). A repeated-measures one-way ANOVA with a Tukey multiple comparison adjustment was performed to determine

significance. Significance values were indicated as not significant (ns) if $p > 0.05$, * = $p \leq 0.05$. ** = $p \leq 0.01$.

Histology: After electrophysiology and behavior experimentation, mice were heavily anesthetized with avertin (2,2,2-tribromoethanol, Sigma-Aldrich, St. Louis, MO, USA; #T48402) and transcardially perfused with ice-cold phosphate buffered saline (PBS, 1x) followed by ice-cold 4% paraformaldehyde (PFA). The implants were removed, and then the brains were dissected out of the skull. The brains were post-fixed in PFA at 4°C for at least 24 hours. They were then cryoprotected via a sucrose gradient, starting at 15% sucrose in PBS followed by 30% sucrose in PBS. The brains were imbedded in Tissue-Tek O.C.T. Compound (Sakura, Torrance, CA, USA), frozen, and then sliced on a cryostat to produce 40 µm thickness sections. The tissue sections were washed in PBS, mounted onto slides, and allowed to dry on the slide for at least 2 hours. The sections were put in a xylene and ethanol series with ~2 min per submersion in the following order: 100% xylene, 100% xylene, 100% ethanol, 100% ethanol, 90% ethanol, 70% ethanol. The sections were then placed in water for ~2 min and then submerged in cresyl violet solution until the stain was sufficiently dark. They were then dehydrated by reversing the series with ~30 s per submersion. Finally, Cytoseal XYL mounting media (Thermo Scientific, Waltham, MA, USA, #22-050-262) and a cover slip were placed on the slides. The slides were allowed to dry in a fume hood before imaging. Photomicrographs were obtained using a Leica DM4000 B LED microscope equipped with a Leica DMC 2900 camera and Leica Application Suite X (LAS X) software. Images were corrected for brightness and contrast using Adobe Photoshop (Adobe Systems, San Jose, CA, USA). Figure panels were made using Adobe Illustrator software.

Acknowledgements
This work was supported by Baylor College of Medicine (BCM), Texas Children's Hospital, The Hamill Foundation, and the National Institutes of Neurological Disorders and Stroke (NINDS) R01NS100874, R01NS119301, and R01NS127435 to RVS. Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number P50HD103555 for use of the Cell and Tissue Pathogenesis Core and *In Situ* Hybridization Core (the BCM IDDRC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. MEvdH was supported by a postdoctoral award from the Dystonia Medical Research Foundation (DMRF) and by the K99NS130463. A portion of the data were collected when AMB was supported by F31NS101891.

Author contributions
Conceptualization: MEvdH, AMB, RVS. Methodology: MEvdH, AMB, RVS. Software: MEvdH, AMB. Validation: MEvdH, AMB. Data Analyses: MEvdH, AMB, RVS. Investigation: MEvdH, AMB, DJK, RVS. Data curation: MEvdH, AMB, DJK. Writing – Original Draft: MEvdH, AMB, RVS. Writing – Review & Editing: MEvdH, AMB, RVS. Visualization: MEvdH, AMB. Supervision: RVS. Project Administration: MEvdH, AMB. Funding acquisition: MEvdH, AMB, RVS.

Figure 1. Supervised classifier model predicts mouse phenotype based on spike signatures. **A.** Schematic of network changes causing motor impairments in mouse models for ataxia, dystonia, and tremor. Dotted lines indicate lack of neurotransmission. Color-coded lines indicate primary affected cell type. **B**. Example of spike firing rate averaged over previous 50 ms at each occurring spike for the 5 s spike train in **B'**. **B''** 1 s spike train for the duration indicated in the square box in **B'**. **C.** Histograms of instantaneous firing rate (ISI^{-1}) of the full 30 s spike train used in the classification model. **D.** Classifier model based on training data set (control: n = 29 cells, N = 9 mice; ataxia: n = 19, N = 5; dystonia: n = 19, N = 8; tremor: n = 21, N = 6). **E.** Predicted phenotype of mice based on spike properties in validation data set (control: n = 30 cells, N = 9 mice; ataxia: n = 18, N = 5; dystonia: n = 17, N = 9; tremor: n = 18, N = 5). Categories on X-axis indicate the origin of the recorded neurons. **F.** and **G.** Scatterplot of spike train parameters used to classify neural signatures. Colored boxes indicate the predicted phenotype, colors of circles indicate the origin of the recorded neurons.
PC = Purkinje cell; CN = cerebellar nuclei; IO/CF = inferior olive/climbing fiber.

Figure 2. Spike signatures are shared across mouse models with similar phenotypes, but different etiologies. **A-H** 5 s example spike trains (and 1 s inset) of representative cerebellar nuclei neurons recorded in each mutant mouse model. **A.** Control mice were littermates to each of the respective experimental groups (N = 19 mice, 84 cells). **B**. Ealy-onset, 4 month-old, ataxic *Atxn1*^{154Q} mice (N = 4, n = 24). **C.** Mildly dystonic *Pdx1*^{Cre}*;Vglut2*$^{fl/fl}$ mice (N = 5, n = 21). **D.** Mildly tremoring *Thap1*$^{+/-}$ mice (N = 6, n = 29). **E.** Late-onset, 10-12 month-old, ataxic *L7*^{Cre}*;Ank1*$^{fl/fl}$ mice (N = 3, n = 14). **F.** Severely dystonic ouabain infusion mice (N = 4, n = 20). **G.** Severely ataxic, dystonic, and tremoring *Car8*$^{wdl/wdl}$ mice (N = 9, n = 19). **H.** Severely ataxic and dystonic *Car8*$^{wdl/wdl}$ mice (N = 6, n = 15). **I.** Predicted phenotype based on the spike activity of the neurons within each data set **J**. Proportion of predicted spike signatures in each of the mouse models on the X-axis. **I, J** are based on classifier model in Figure 1D.

Figure 3. Spike signatures in cerebellar nuclei neurons can be induced by specific stimulation paradigms of Purkinje cells. **A.** Schematic of experimental setup with recordings in awake, head-fixed mice. **B.** Optopatcher recordings of cerebellar nuclei neurons. The opsin is expressed in Purkinje cells (pink) and recordings of nuclei neurons are performed. **C.** Example validation that light stimulation of inhibitory Purkinje cells (blue bars) inhibits nuclei neurons during light stimulation. The lower trace is a blown-up view of the boxed area in the upper trace. **D.** Example of spike firing rate averaged over previous 50 ms at each occurring spike for the 5 s spike train in **D'**. **D''** 1 s spike train for the duration indicated in the square box in **D'**. Blue bars indicate light stimulation and are specific for ataxia, dystonia, and tremor (see methods for light stimulation parameters). All example traces originate from the same nuclei neuron, indicating that the cell's spike signature can change depending on the light stimulation paradigm. **E.** Proportion of cells of each predicted spike signature during each of the light stimulations based on classifier model in Figure 1D. Control: n = 11 cells, N = 4 mice; ataxia: n = 9, N = 3; dystonia: n = 7, N = 3; tremor n = 9, N = 4. PC = Purkinje cell; CN = cerebellar nuclei; IO/CF = inferior olive/climbing fiber.

Figure 4. Induced spike signatures elicit distinct cerebellar phenotypes. **A.** Schematic of external view of bilateral optical fiber implant. **B.** Schematic of a coronal section from a mouse cerebellum with bilateral optic fiber implants directed towards the cerebellar nuclei. FN = fastigial nucleus. IN = interposed nucleus. DN = dentate nucleus. **C.** Photomicrograph of a Nissl-stained coronal section from a mouse cerebellum that had been implanted with optic fibers. Arrows = optic fiber tracks. Dotted lines surround the cerebellar nuclei indicated in **B**. Scale = 1 cm. **D.** Schematic of a mouse with bilateral optic fiber implants freely moving in a tremor monitor. **E-K.** Data associated with gait measurements. **E-H.** Example footprints from a single mouse before stimulation (baseline, **E**) and during ataxia (**F**), dystonia (**G**), and tremor stimulation (**H**). Scale = 1 cm. **I-K.** Measurements of gait including the length of the hindpaw stride (**I**), forepaw stride (**J**), and distance between the hind and forepaws (**K**). N = 8. * = $p \leq 0.05$. ** = $p \leq 0.01$. **L**. Example images of phenotypes associated with dystonia. 1 = erect tail. 2 = high stepping. 3 = kinked

tail. 4 = hyperextension of the limbs. 5 = splayed toes. **M.** Dystonia rating of mice before stimulation and during stimulation with each paradigm. N = 8. * = $p \leq 0.05$. **N.** Tremor signals detected via tremor monitor from a mouse before and during stimulation with each paradigm. Horizontal scale = 1 sec. Vertical scale = 50 mV. **O.** Population average power spectrums of tremor. Solid line = mean power. Shaded region = SEM. **P.** Peak tremor power of mice before and during stimulation with each paradigm. N = 8. * = $p \leq 0.05$. ** = $p \leq 0.01$. **Q-S.** 2-D comparisons of gait (hind to fore distance), dystonia (rating), and tremor (peak power) measurements from all mice. N = 8. **T.** 3-D plot of data in **Q-S**.

Video 1: Optogenetically-induced behavioral responses in head-fixed mice. Subtle features of ataxia, dystonia, and tremor can be induced in head-fixed mice by initiating each specific spike signature. In this experiment, the optopatcher recording and stimulation allowed for tracking the inductions of spike signatures in single-units and the subsequent presentation of the disease-associated behaviors. In this video, we show an example of a single trace of multiunit activity recorded from the cerebellar cortex (putative Purkinje cell activity) with the voltage of the trace played as audio in response to all stimulation paradigms and a video of the mouse's behavioral response to each paradigm during the recording.

Video 2. Optogenetically-induced spike signatures result in severe cerebellar phenotypes. Examples of a single freely moving mouse's behavior at baseline and in response to bilateral induction of spike signatures.

Video 3. Optogenetically-induced spike signatures affect gait. Examples of a single mouse's gait within a footprinting corridor at baseline and in response to bilateral induction of spike signatures.

Supplemental Figure 1. Spike train signatures are shared across mouse models with similar phenotypes, but different etiologies. **A.** Optopatcher recordings of a Purkinje cell. The opsin is expressed in Purkinje cells (pink). **B.** Example validation that light stimulation (blue bars) excites Purkinje cells. The pink lines indicate climbing fiber induced complex spikes, further confirming that these recordings are from a Purkinje cell. The lower trace is a blown-up view of the boxed area in the upper trace. **C.** Example of spike firing rate averaged over previous 50 ms at each occurring spike for 5 s spike train in **C'**. **C''** 1 s spike train for the duration indicated in the square box in **C'**. Blue bars indicate light stimulation and are specific for ataxia, dystonia, and tremor (see methods for light stimulation parameters). All example traces originate from the same nuclei neuron, indicating that the cells spike signature can change depending on the light stimulation paradigm. PC = Purkinje cell; CN = cerebellar nuclei; IO/CF = inferior olive/climbing fiber.

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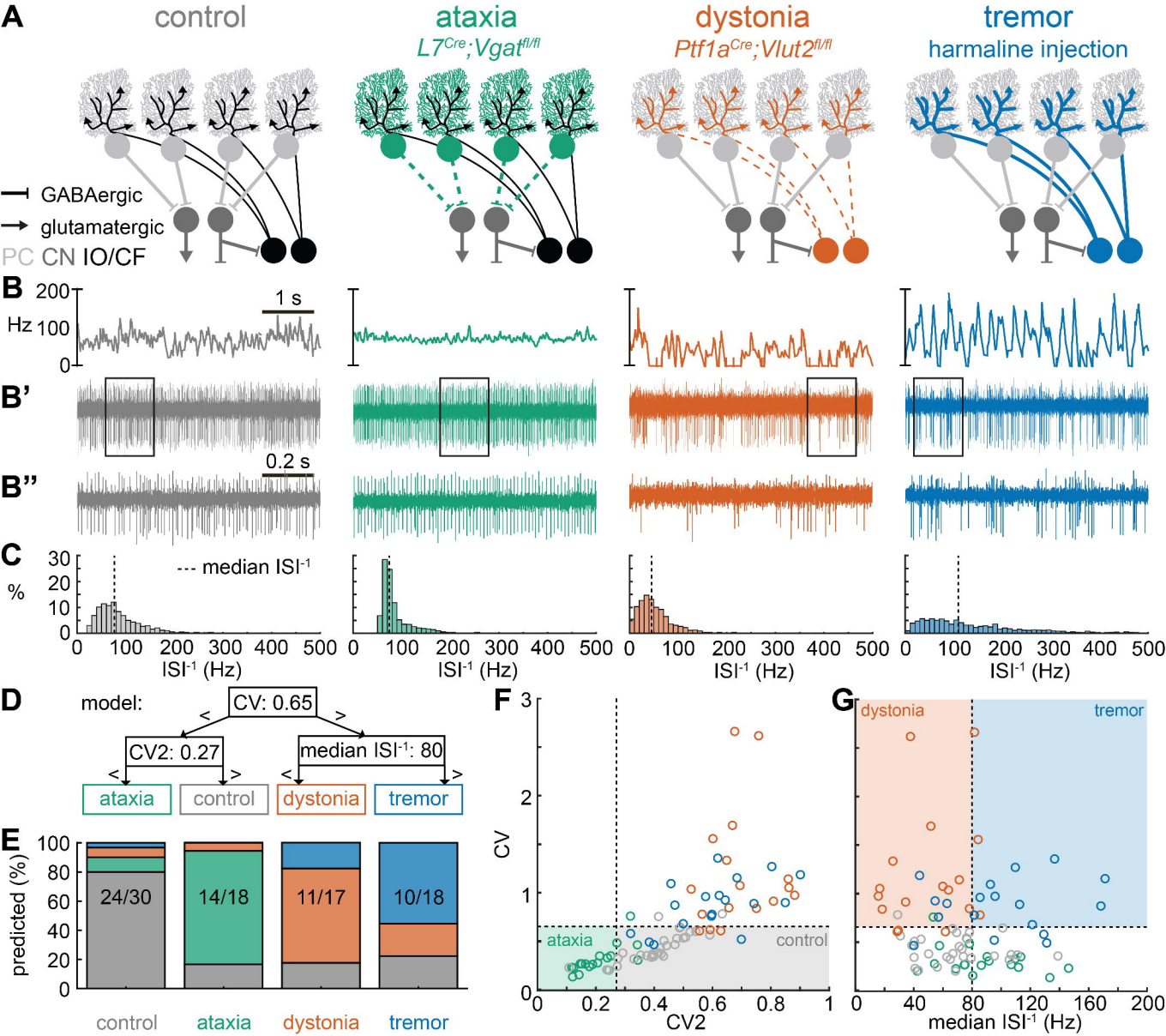
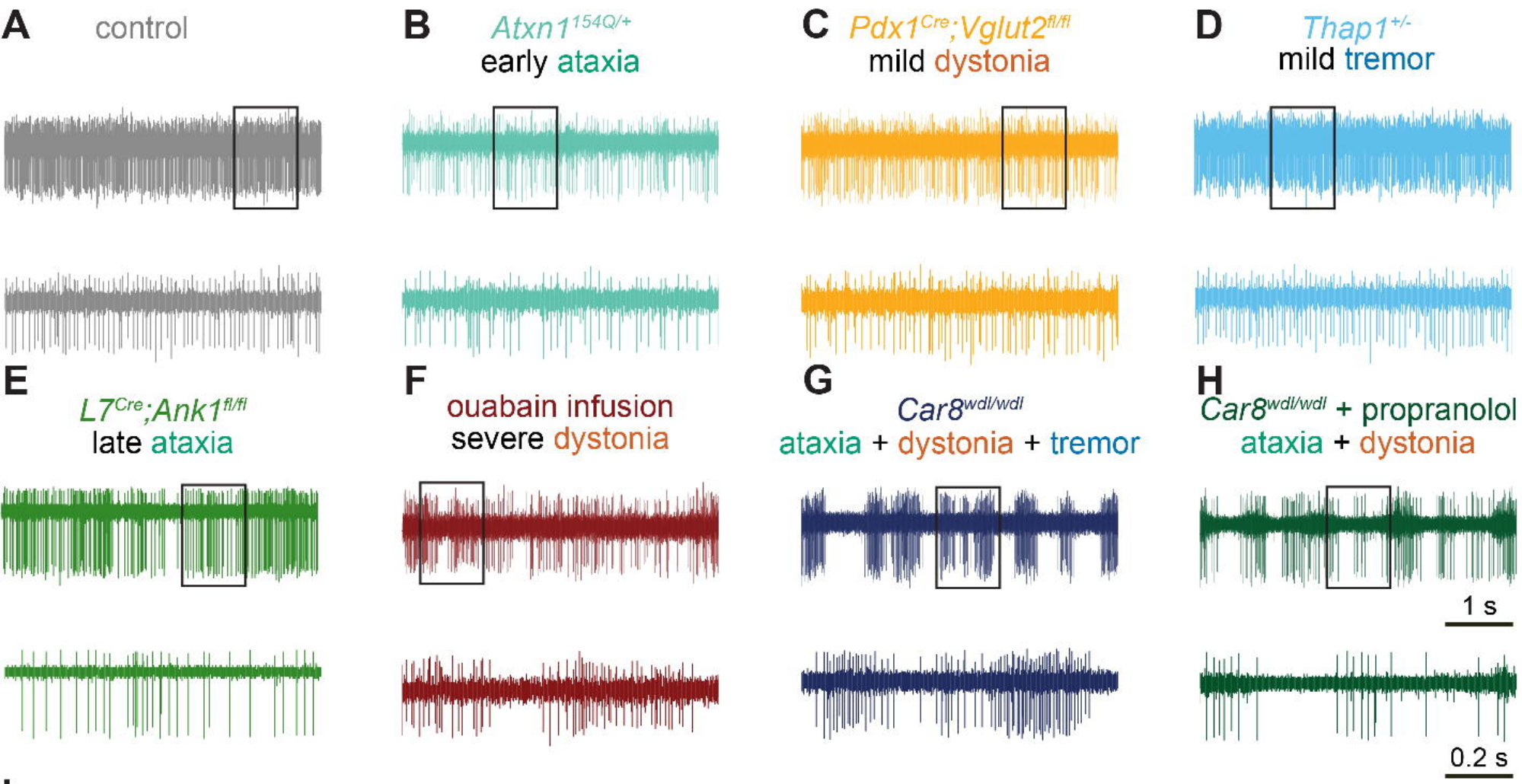
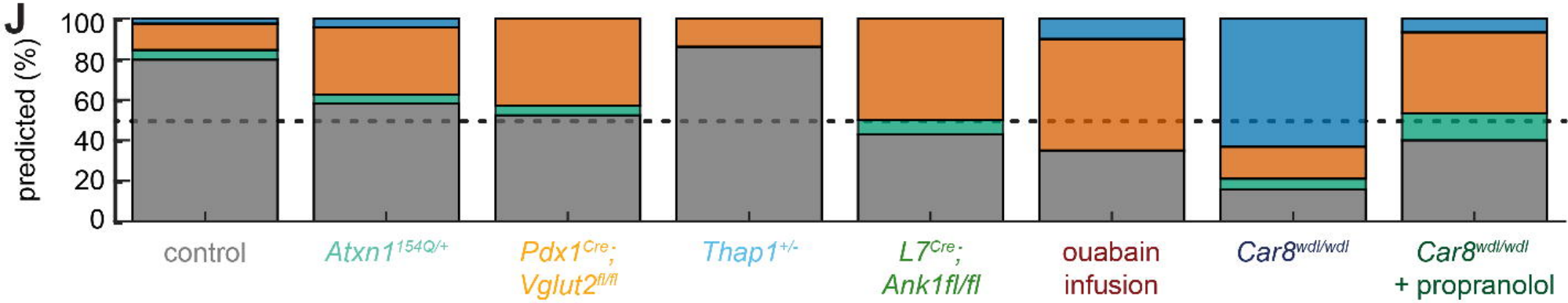
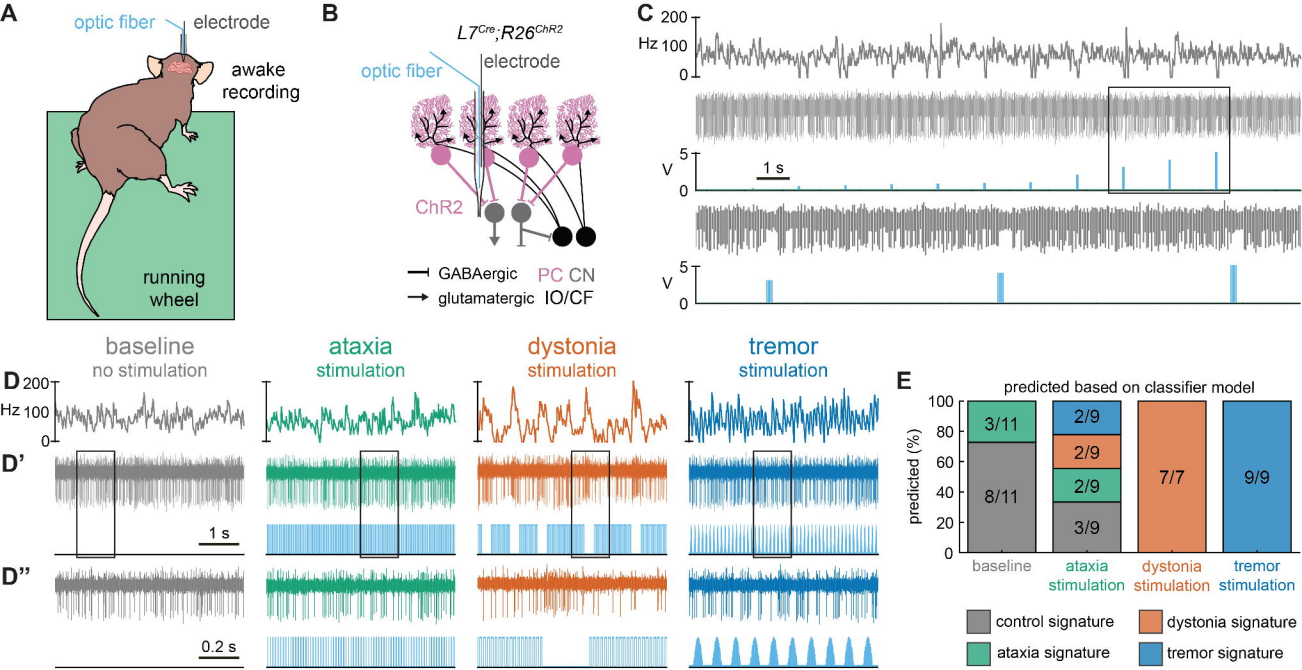

A
control
ataxia
L7Cre;Vgatfl/fl
dystonia
Ptf1aCre;Vlut2fl/fl
tremor
harmaline injection
GABAergic
glutamatergic
PC CN IO/CF
B
200
Hz 100
0
1 s
B'
B''
0.2 s
C
30
20
10
0
%
--- median ISI-1
0 100 200 300 400 500
ISI-1 (Hz)
D
model:
CV: 0.65
CV2: 0.27
median ISI-1: 80
ataxia
control
dystonia
tremor
E
100
80
60
40
20
0
predicted (%)
24/30
14/18
11/17
10/18
control
ataxia
dystonia
tremor
F
3
2
1
0
CV
ataxia
control
0 0.2 0.4 0.6 0.8 1
CV2
G
dystonia
tremor
0 40 80 120 160 200
median ISI-1 (Hz)

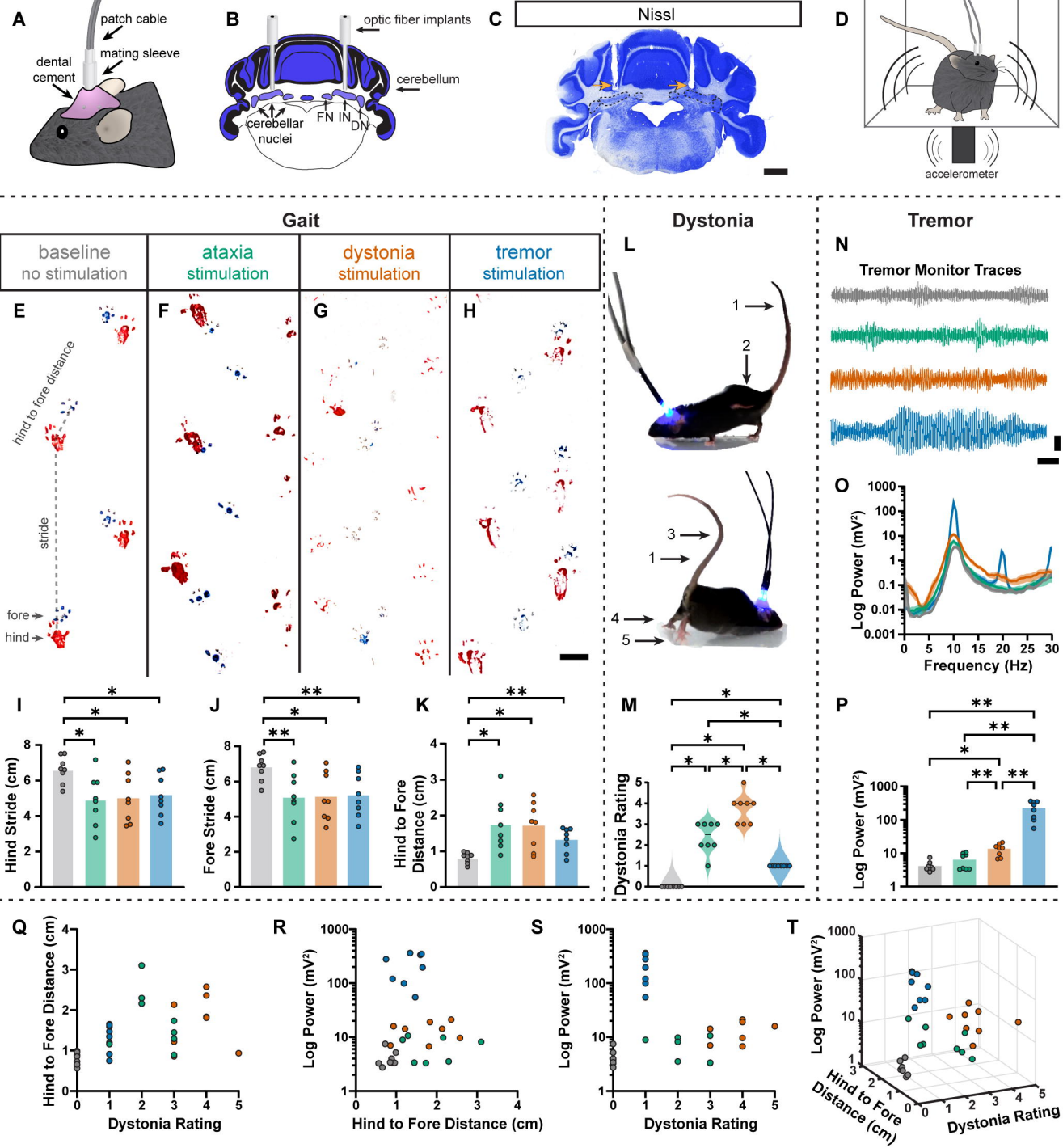


I

control	67/84	14/24	11/21	25/29	6/14	7/20	3/19	6/15
ataxia	4/84	1/24	1/21	0/29	1/14	0/20	1/19	2/15
dystonia	11/84	8/24	9/21	4/29	7/14	11/20	3/19	6/15
tremor	2/84	1/24	0/21	0/29	0/14	2/20	12/19	1/15




A
optic fiber
electrode
awake recording
running wheel
B
L7Cre;R26ChR2
optic fiber
electrode
ChR2
GABAergic
glutamatergic
PC CN
IO/CF
C
Hz
V
1 s
baseline
no stimulation
ataxia
stimulation
dystonia
stimulation
tremor
stimulation
D
D'
D''
1 s
0.2 s
E
predicted based on classifier model
predicted (%)
3/11
8/11
2/9
2/9
2/9
3/9
7/7
9/9
baseline
ataxia stimulation
dystonia stimulation
tremor stimulation
control signature
ataxia signature
dystonia signature
tremor signature


A
patch cable
mating sleeve
dental cement
B
optic fiber implants
cerebellum
cerebellar nuclei
FN
IN
DN
C
Nissl
D
accelerometer
Gait
baseline
no stimulation
ataxia
stimulation
dystonia
stimulation
tremor
stimulation
E
F
G
H
hind to fore distance
stride
fore
hind
Dystonia
L
1
2
3
1
4
5
Tremor
N
Tremor Monitor Traces
O
Log Power (mV²)
Frequency (Hz)
I
Hind Stride (cm)
J
Fore Stride (cm)
K
Hind to Fore Distance (cm)
M
Dystonia Rating
P
Log Power (mV²)
Q
Hind to Fore Distance (cm)
Dystonia Rating
R
Log Power (mV²)
Hind to Fore Distance (cm)
S
Log Power (mV²)
Dystonia Rating
T
Log Power (mV²)
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Dystonia Rating