

DYSTONIA

Pathophysiology of Dyt1-Tor1a dystonia in mice is mediated by spinal neural circuit dysfunction

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Dystonia, a neurological disorder defined by abnormal postures and disorganized movements, is considered to be a neural circuit disorder with dysfunction arising within and between multiple brain regions. Given that spinal neural circuits constitute the final pathway for motor control, we sought to determine their contribution to this movement disorder. Focusing on the most common inherited form of dystonia in humans, DYT1-TOR1A, we generated a conditional knockout of the torsin family 1 member A (*Tor1a*) gene in the mouse spinal cord and dorsal root ganglia (DRG). We found that these mice recapitulated the phenotype of the human condition, developing early-onset generalized torsional dystonia. Motor signs emerged early in the mouse hindlimbs before spreading caudo-rostrally to affect the pelvis, trunk, and forelimbs throughout postnatal maturation. Physiologically, these mice bore the hallmark features of dystonia, including spontaneous contractions at rest and excessive and disorganized contractions, including cocontractions of antagonist muscle groups, during voluntary movements. Spontaneous activity, disorganized motor output, and impaired monosynaptic reflexes, all signs of human dystonia, were recorded from isolated mouse spinal cords from these conditional knockout mice. All components of the monosynaptic reflex arc were affected, including motor neurons. Given that confining the *Tor1a* conditional knockout to DRG did not lead to early-onset dystonia, we conclude that the pathophysiological substrate of this mouse model of dystonia lies in spinal neural circuits. Together, these data provide new insights into our current understanding of dystonia pathophysiology.

INTRODUCTION

Neural circuits that control movement are distributed across the neuraxis and are composed of multiple interconnected loops involving the cerebral cortex (1), basal ganglia (2, 3), thalamus (4), cerebellum (5), brainstem (6), and spinal cord (7). Whereas each of these loops has its own function, it is the collaboration of the ensemble that ultimately produces functional movement and hence behavior. When dysfunction develops within or between these loops, movement disorders arise.

Dystonias are common movement disorders and are characterized by involuntary sustained muscle contractions across multiple muscle groups, manifesting as abnormal posture and disorganized movements (8, 9). The irregular muscle activity leading to these hallmark postures and movements bears three main neurophysiological signatures: (i) spontaneous muscle contraction at rest (10); (ii) excessive, sustained contractions during voluntary movements often involving cocontractions of antagonistic muscles, which may lead to pain in addition to dysfunctional movement (10, 11); and (iii) altered involuntary sensory-motor reflexes (12, 13). Nonetheless, the neural circuit dysfunction that underlies the pathophysiology of dystonia is not well understood.

The first link among motor control, movement disorders, and the basal ganglia—a cluster of subcortical nuclei—was drawn in the 1600s (14). Thereafter, multiple movement disorders were subsequently classified as basal ganglia syndromes throughout the 1800s and early 1900s, including Parkinson's disease, Huntington's disease, and dystonia (15–17). In dystonia, however, limited pathology has been found in the basal ganglia in either humans (18) or animals (19). Moreover, there is a multi-month lag between acquired injury (such as stroke) of the basal ganglia and development of dystonia (20) as well as a similar lag between deep brain stimulation (DBS) of the basal ganglia and alleviation of symptoms (21). Furthermore, not all basal ganglia lesions give rise to dystonia (16), and attempts to genetically manipulate basal ganglia nuclei to produce mouse models of dystonia have not been successful (22). To accommodate these findings, dystonia is now commonly considered to be a circuitopathy comprising multiple interconnected brain regions involved in movement, including the basal ganglia, thalamus, cerebellum, and cortex (15).

All output originating from these regions is mediated via motor neurons in the brainstem and spinal cord that send direct projections to muscles to produce coordinated movements. Spinal motor circuits provide key input to motor neurons; these circuits produce and concatenate the basic syllables of limb movement that are disorganized in dystonia: muscle contractions across joints, within limbs, and between limbs (23). Given that the spinal cord is the final common pathway for motor control and that dystonia is defined by its abnormal muscle contractions and movement disorganization (10), we sought to determine whether spinal cord dysfunction could be responsible for the pathogenesis of the clinical signs of dystonia.

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In this study, we focused on the most prevalent genetic form of dystonia: early-onset generalized torsional dystonia, or DYT1-*TOR1A*, which is commonly caused by an in-frame deletion of three base pairs in exon 5 of the torsin family 1 member A (*TOR1A*) gene (24). We made a mouse model of DYT1-*TOR1A* dystonia that confines *Tor1a* deletion to spinal cord and dorsal root ganglion (DRG) neurons. We show that these mice develop functional and physiological signs that mirror those seen in human DYT1-*TOR1A* dystonia and used these mice to map spinal motor circuit dysfunction. Confining *Tor1a* deletion to DRG neurons did not reproduce the phenotype. We conclude that spinal-restricted deletion of *Tor1a* reproduces the pathophysiology of the human condition.

RESULTS

Restricting *Tor1a* deletion to spinal circuits and DRGs

Unraveling the specific contributions of spinal cord dysfunction to movement disorganization in Dyt1-*Tor1a* dystonia requires site specificity in *Tor1a* deletion; that is, spinal circuits must be directly affected while supraspinal centers are spared. To this end, we used the established Cdx2::FlpO transgenic mouse model as our genetic entry point to manipulating *Tor1a* in spinal circuits, because flipase expression is restricted to the developing spinal cord and DRG (25). Drawing inspiration from a prevailing *Tor1a*-flox approach (26–28), we developed a new *Tor1a*-f^{rt} mouse in which exons 3 to 5 are flanked by f^{rt} sites (Fig. 1A and fig. S1A). Through multigenerational breeding of *Tor1a*-f^{rt} with Cdx2::FlpO mice, we generated a caudal-restricted biallelic “double” conditional knockout (d-cko) of *Tor1a* (fig. S1, B and C). Probing for *Tor1a* and torsinA protein expression in brains, lumbar spinal cords, and DRGs confirmed the site specificity of this approach. When compared with FlpO-negative littermate controls (Cdx2::wt; *Tor1a*^{f^{rt}/f^{rt}}), caudal-restricted *Tor1a* d-cko (Cdx2::FlpO; *Tor1a*^{f^{rt}/f^{rt}}) mice showed normal *Tor1a*-torsinA expression in the brain (and heart and liver) but a virtual absence in lumbar spinal cords and DRGs (Fig. 1, B and C; and fig. S1, D to F), thus validating the site specificity of our strategy.

Nuclear envelope pathology in *Tor1a*-deleted spinal and DRG neurons

We next screened for the ultrastructural signature of torsinA dysfunction, nuclear envelope (NE) malformations. Canonical torsinA expression in neurons is distributed throughout the endoplasmic reticulum and NE, but in loss-of-function mutations, torsinA aberrantly accumulates in the NE (29). Morphologically, this can lead to outer nuclear membrane protrusions that balloon into the perinuclear space, where they are released as vesicles (27, 30). We found that littermate control spinal neurons had normal, well-defined, closely apposed nuclear bilayers with occasional nucleoplasmic reticulations decorating the nuclei (Fig. 1, D to F, J, and K, arrowheads). In contrast, *Tor1a*-deleted lumbar spinal neurons were replete with NE abnormalities. In the dorsal horns, there were groups of spinal neurons that appeared normal (Fig. 1G), exhibited early signs of NE budding with sparse vesicle accumulation (Fig. 1H), or showed a vesicle-packed perinuclear space with overt separation of the nuclear membranes (Fig. 1I). In contrast, in the ventral horns, almost all spinal neurons screened were affected, with the perinuclear space filled with NE-derived

vesicles (Fig. 1L). Multiple vesicles often budded from one protrusion point of the inner nuclear membrane (Fig. 1M), with signs of electron-dense nucleolar content filling the vesicles and being released (Fig. 1N). Large-area ultrastructural analysis via backscatter scanning electron microscopy of all contiguous spinal neurons embedded within hemiscord slices corroborated the ventrodorsal gradient in NE severity. Of the 2711 spinal neurons screened in the L4 and L5 segments, about 60 ± 6% showed ultrastructural abnormalities consistent with torsinA loss of function. In contrast, 8.0 ± 5.0% of the DRG neurons screened in these same segments were affected (fig. S1, H to L and W to Y), suggesting that spinal neurons are particularly vulnerable to *Tor1a* dysfunction. Moreover, ultrastructural screening of basal ganglia neurons did not show aberrant NE budding or vesiculation (fig. S1, M to V). Together, these data confirm that this new mouse model confines the *Tor1a* d-cko to spinal and DRG neurons, with spinal neurons showing ultrastructural signs of torsinA loss of function. The model will henceforth be referred to as spinal *Tor1a* d-cko mice.

Spinal-restricted *Tor1a* leads to severe early-onset generalized dystonia

In humans, severe DYT1-*TOR1A* is defined by the early-onset generalized spread of disorganized movements, usually beginning in a lower extremity and then spreading to the trunk and upper limbs (31). The signs stabilize at or below the neck, usually sparing cranial muscle function (32). We discovered similarities between the signs of severe DYT1-*TOR1A* in humans and those in spinal *Tor1a* d-cko mice (Fig. 2, A to H). The motor impairments in spinal *Tor1a* d-cko mice emerged early, within the first 1 to 3 days after birth, manifesting caudally as hindlimb hyperextension (Fig. 2A and movie S1). These signs spread bilaterally to affect both lower extremities (Fig. 2B) by postnatal day 5 (P5). With increasing age, the motor impairments spread rostrally such that by P7 to P9, there were clear signs of pelvis, trunk, and forelimb dysfunction, with the forelimbs abnormally extended forward and minimal body weight support (Fig. 2, C and D). By P11, the motor signs became fixed at or below the head, sparing orofacial movements. Stepping was impaired as indicated by excessive hindlimb hyperextension with minimal flexion (Fig. 2E). Hind paw clasping and truncal torsion occurred during tail suspension (Fig. 2F and movie S2), a test commonly used in Dyt1-*Tor1a* dystonia animal models to uncover latent dystonic-like behaviors (33, 34). By P19 to P21, spinal *Tor1a* d-cko mice were severely dystonic, with profound abnormal posturing, disorganization of limb movements (Fig. 2G and movie S3), and bouts of debilitating truncal torsion (Fig. 2H and movie S4). Similar to severe DYT1-*TOR1A* in humans, postures were abnormal (movie S5), and movements were disorganized, jerky, and tremulous in spinal *Tor1a* d-cko mice (movie S6).

Given that Cdx2::FlpO directs recombination to both spinal and DRG neurons (25), we set out to determine whether *Tor1a* dysfunction confined to DRGs alone (termed “DRG *Tor1a* d-cko mice”) leads to early-onset generalized dystonia. Leveraging an analogous multigenerational breeding strategy, we confined the biallelic knockout of *Tor1a* exons 3 to 5 to DRG neurons using Advillin-cre (35) and *Tor1a*-flox mice (34). After confirming that *Tor1a* was deleted from DRGs and spared in the spinal cord using quantitative polymerase chain reaction (qPCR; table S1), postnatal video recordings were performed. In contrast to the spinal *Tor1a* d-cko

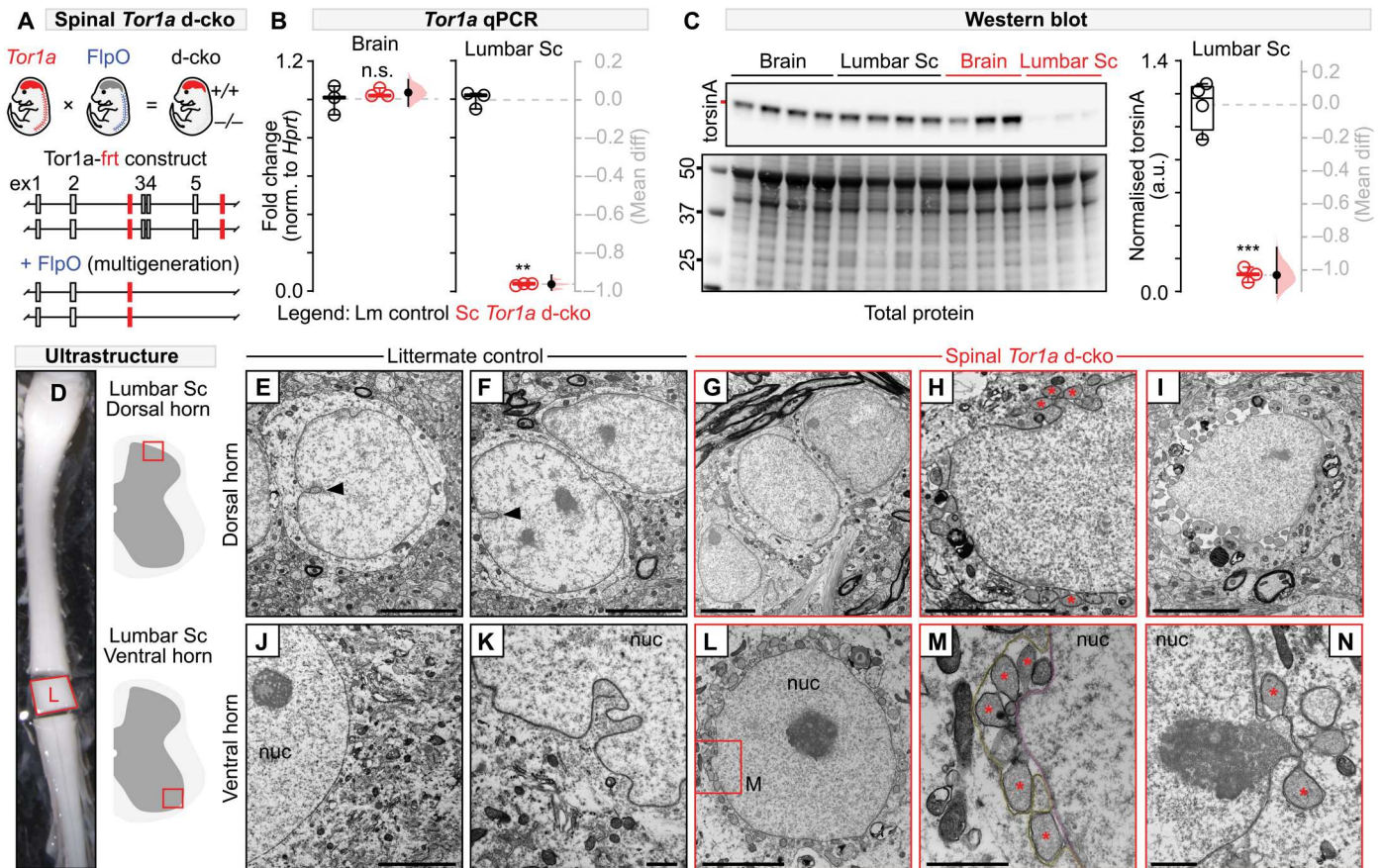


Fig. 1. Characterization of the spinal-restricted d-cko of *Tor1a*. (A) Shown is a schematic of the genetic strategy used to restrict *Tor1a* deletion to the mouse spinal cord. (B) qPCR was used to quantify *Tor1a* expression in brains and lumbar spinal cords of spinal *Tor1a* d-cko mice and littermate controls (***P* < 0.001, independent *t* test; *n* = 3 P18 control and *n* = 3 P18 spinal *Tor1a* d-cko). n.s., not significant. (C) Western blots are shown for torskA (~37.5 kDa) expression in mouse brains and lumbar spinal cords (***P* < 0.0001; *n* = 4 P18 control and *n* = 3 spinal *Tor1a* d-cko). a.u., arbitrary units. (D) Lumbar spinal cords were isolated for ultrastructural analyses of dorsal (E to I) and ventral horn (J to N) spinal neurons (*n* = 4 P18 control and *n* = 4 spinal *Tor1a* d-cko mice) using electron microscopy. (E and F) Dorsal and (J and K) ventral horn neurons of littermate controls showed normal nuclear membrane morphology with occasional nuclear invaginations (black arrowheads). Dorsal (G to I) and ventral (L to N) neurons in spinal *Tor1a* d-cko mice showed NE abnormalities, including perinuclear accumulation of vesicles (asterisks) and separation between the inner (M) (magenta) and outer nuclear membranes (M) (yellow). nuc, nucleus. Scale bars, 5 (E to J and L) and 1 μm (K, M, and N). Group data are shown (box plots) with individual values overlaid (circles) and mean differences (Gardner-Altman estimation plots). [Also see table S1 and fig. S3.]

model, DRG *Tor1a* d-cko mice did not develop early-onset generalized torsional dystonia (movie S7). That is, it is *Tor1a* deletion in the spinal cord that leads to the dystonic phenotype.

After establishing that spinal-restricted *Tor1a* d-cko causes an early-onset generalized movement disorder, we set out to unambiguously define the spatiotemporal window of the dystonic-like phenotype. Five external raters experienced with mouse behavior were recruited to provide unbiased analyses of postnatal sensory-motor development in littermate controls versus spinal *Tor1a* d-cko mice (Fig. 2I) while blinded to the study design, disease model, mutation, and anticipated motor impairments. Raters assessed postnatal video recordings (P1 to P13) and selected, via a unidirectional online test, whether the mouse was a “control” or “mutant,” the latter prompting a follow-up question to select the body regions affected. The unbiased external analysis corroborated our internal findings. At P1, the accuracy rate (proportion of correct observations) in the unbiased detection of spinal *Tor1a* d-cko mice was >60% (Fig. 2J). Throughout postnatal maturation, the accuracy rate steadily increased until it reached 100% at P7, with any subsequent inaccuracy due to false

positives (P1 to P6: 73% sensitivity and 88% specificity and P7 to P13: 100% sensitivity and 98% specificity). A clear spatiotemporal pattern emerged from these unbiased assessments, with motor impairments noted early in the hindlimbs and then spreading rostrally to affect the pelvis, trunk, and forelimbs, with the head minimally affected (Fig. 2K). Thus, *Tor1a* dysfunction confined to spinal circuits causes an overt movement disorder that recapitulates the spatiotemporal motor signs of severe early-onset generalized dystonia.

The disordered postures and movements observed in severe DYT1-TOR1A in humans are defined by (i) persistent involuntary electromyogram (EMG) activity at rest (10) and (ii) disorganized muscle activity during voluntary movements, often including antagonistic cocontractions (10, 11). To test whether the loss of spinal *Tor1a* causes similar aberrant muscle activity signs, we performed acute EMG recordings from the antagonistic tibialis anterior and gastrocnemius hindlimb muscles in preweaned wild-type control and spinal *Tor1a* d-cko mice (Fig. 2M).

At rest, there was little to no evidence of spontaneous muscle activity in control mice (Fig. 2M). Conversely and similar to severe

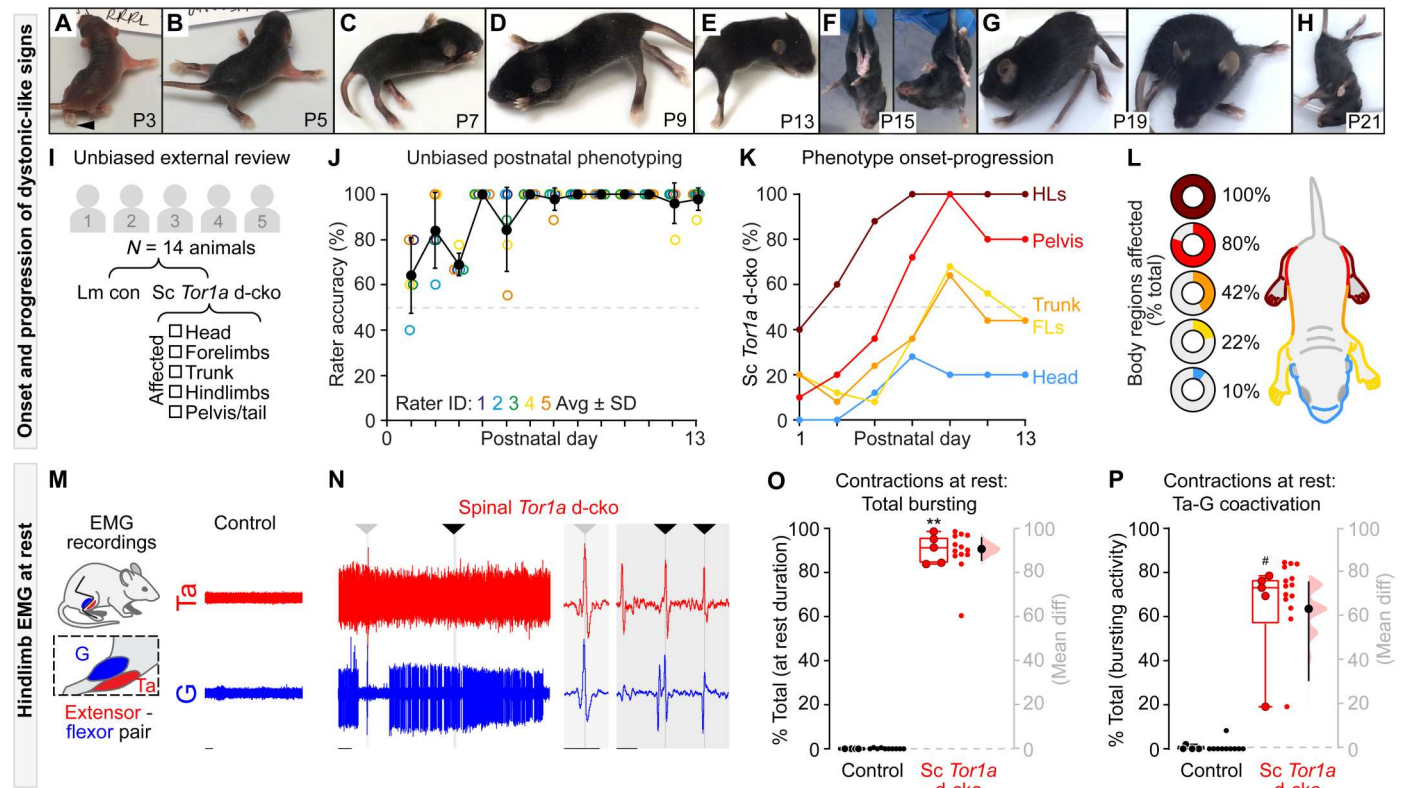


Fig. 2. Spinal-restricted *Tor1a* d-cko leads to early-onset, caudo-rostral progression of movement disorganization marked by abnormal muscle activity. (A to H) Shown are representative still images of onset-progression of dystonic-like signs in spinal *Tor1a* d-cko mice. **(I)** Experimental design for unbiased external review of phenotype ($n = 8$ control and $n = 6$ spinal *Tor1a* d-cko mice). Lm con, littermate control; Sc *Tor1a* d-cko, spinal *Tor1a* d-cko. **(J)** Rater accuracy in identifying genetically confirmed spinal *Tor1a* d-cko mice. **(K)** Spatiotemporal progression of the spinal *Tor1a* d-cko phenotype. HLs, hindlimbs; FLs, forelimbs. **(L)** Depicted are the body regions affected in spinal *Tor1a* d-cko mice. **(M to P)** EMG recordings from gastrocnemius (G) and tibialis anterior (Ta) ($n = 6$ P17 and P19 spinal *Tor1a* d-cko mice). **(M)** Representative EMG activity during rest from a P18 wild-type control mouse. **(N)** Spinal *Tor1a* d-cko mice showed excessive spontaneous EMG activity at rest [same amplification as **(M)**], including coincident single-unit spikes (arrowheads; expanded view in shaded regions). **(O)** Quantification of spontaneous contractions observed at rest ($n = 4$ control and $n = 5$ spinal *Tor1a* d-cko mice). **(P)** Quantification of Ta-G cocontractions during the at-rest spontaneous EMG activity. $**P < 0.0001$, independent t test; $\#P < 0.05$, Mann-Whitney U test. Group data are shown (box plots) with individual mean values overlaid (circles) and mean differences (estimation plots). Dots, raw data, all at-rest epochs analyzed per animal. Scale bars, 1 (M and N) and 0.1 s (N) (shaded regions). [Also see table S1, fig. S2, and movies S1 to S7.]

DYT1-TOR1A in humans, spinal *Tor1a* d-cko mice had excessive spontaneous activity at rest, including prolonged coactive bursting in antagonist muscles (Fig. 2N and fig. S2, B and C) with few periods of quiescence (fig. S2A). More than 90% of the total resting EMG activity was marked by hindlimb muscle activity (Fig. 2O), of which 65% was flexor-extensor coactivation (Fig. 2P), including coactive single units (Fig. 2N, shaded regions). We also noted episodes of whole hindlimb stiffness associated with cocontractions of ankle extensor-flexor muscles (fig. S2, D to K) and a proximal tremulous-like phenotype (movie S6), a phenomenon also reported in people with dystonia (11).

Although we were unable to obtain EMG recordings in control preweaned mice given the high level of activity, we leveraged the limited mobility of spinal *Tor1a* d-cko mice to assess EMG activity during tail suspension, a common litmus test for abnormal body posturing in dystonic rodents (fig. S2L) (33, 34), and volitional locomotion. Tail suspension uncovered a spectrum of EMG patterns ranging from alternation between flexor-extensor bursting to large amplitude cocontractions and burst disorganization between tibialis anterior and gastrocnemius that was interspersed with tonic activity and rhythmic cocontractions (fig. S2, M to Q). During

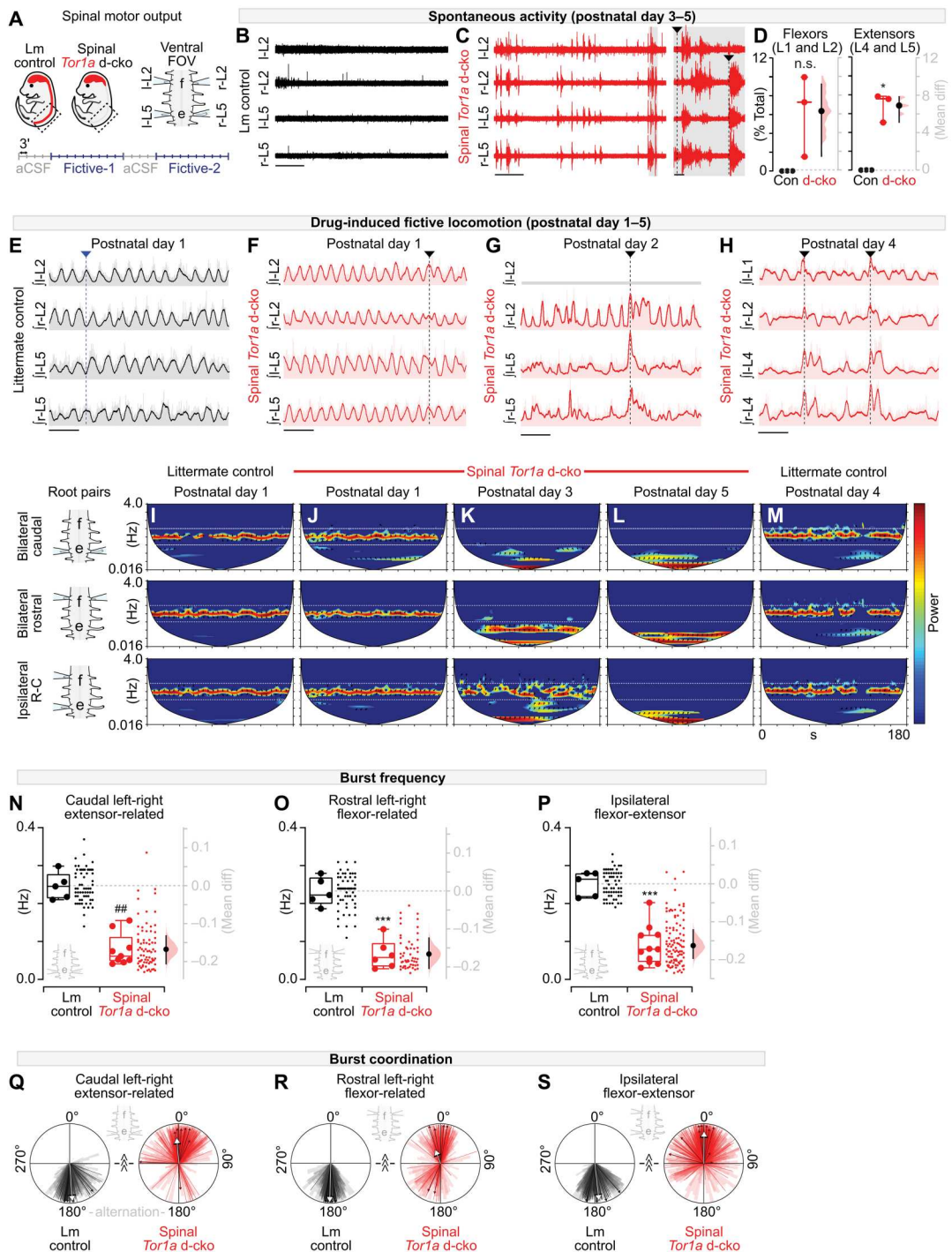
volitional stepping, spinal *Tor1a* d-cko mice showed hindlimb muscle activity with multiple bouts of antagonist cocontractions (fig. S2, R and S). In total, cocontractions of tibialis anterior and gastrocnemius accounted for more than one-third of the bursting activity observed during stepping (fig. S2U). This was an underestimate of the amount of coactivity; when considering single-unit coincident activity in addition to the bursts, the proportion of cocontraction time increased (fig. S2, V and W). Disorganized hindlimb muscle activity was further underscored by a considerable range in frequency and duration of bursts (fig. S2, X and Y). Together, these data reveal that spinal-restricted *Tor1a* dysfunction directly leads to an early-onset generalized dystonic-like movement disorder defined by persistent spontaneous muscle activity at rest and excessive cocontractions during rest and voluntary movements.

Excessive, disorganized motor output in mice with spinal deletion of *Tor1a*

We next determined whether *Tor1a*-deleted spinal circuits were the principal source of excessive spontaneous activity and disorganized motor output. Given that the lower extremities are a primary site for disease onset in severe DYT1-TOR1A and spinal *Tor1a* d-cko mice,

Fig. 3. *Tor1a*-deleted spinal circuits produce excessive spontaneous activity and disorganized motor output.

(A) Shown is a schematic of the experimental design for motor output recordings (P1 to P5 recordings). FOV, field of view. (B and C) Representative traces (at same amplification) of spontaneous activity at "rest" in artificial cerebrospinal fluid (aCSF). (D) Quantification of spontaneous activity (P3 to P5: 800 to 980 s per preparation, $n = 3$ control versus $n = 3$ d-cko mice; $*P < 0.05$, independent t test with Welch's correction). (E) Representative traces of rhythmic, coordinated bursting during drug-induced fictive locomotion in control. Dashed line, alternating bursts at left-right L2, left-right L5, and ipsilateral L2 to L5. (F to H) Drug-induced fictive locomotion in spinal *Tor1a* d-cko mice. Dashed lines, burst discoordination. Three-channel recording shown in (G). (I to M) Cross-wavelet analysis of frequency power (color, blue-red: 2^7 to 2^{13} a.u.) spectra with phase overlaid (arrows). Horizontal lines: control frequency range. (N to P) Quantification of burst frequency in root pairs assessed in spinal *Tor1a* d-cko ($n = 6$ to 11) versus control ($n = 5$) mice ($^{##}P = 0.001$, Mann-Whitney U test; $^{***}P < 0.0001$, independent t test). Group data are shown (box plots) with individual means overlaid (circles) and mean differences (estimation plots). Dots, raw data, all epochs analyzed per animal. (Q to S) Quantification of burst coordination ($^{***}P < 0.001$, Watson's nonparametric U^2 test). Bold arrows, orientation: mean phase, length (0 to 1): concentration of observations. Group data are overlaid onto total observations from all epochs (wedges) and epoch averages (lines). Scale bars, 30 (B and C) (left), 10, (E to H), and 1 s (C) (right). [Also see table S1 and fig. S3.]



we performed targeted recordings of neural activity intrinsic to the lumbar enlargement, the neural hub for hindlimb motor control (7). Lumbar spinal cords were isolated from P1 to P5 mice, thus eliminating the influence of descending systems on spinal motor output and framing the previously defined window of emerging hindlimb dysfunction. Extracellular electrodes were attached to the caudal and rostral lumbar ventral roots to record electroencephalogram (ENG) activity from extensor- and flexor-related spinal motor pools, respectively (Fig. 3A) (36).

In early postnatal isolated spinal cords, there is often spontaneous ENG activity at rest (that is, in the absence of evoked activity), a transient phenomenon that dissipates until little, if any, activity is present by P3 to P5 (37), as seen in our littermate controls (Fig. 3, B and D). On the other hand, there was spontaneous activity in all isolated spinal cords from P3 to P5 spinal *Tor1a* d-cko mice (Fig. 3D). During much of this spontaneous activity, there was co-activation of antagonistic caudal extensor-related (L5) and rostral flexor-related (L2) motor pools (Fig. 3C, shaded inset). These

results indicate that the spontaneous activity recorded in the EMGs of spinal *Tor1a* d-cko mice in vivo could result from spontaneous activity in the spinal cord circuits.

Spinal circuits can directly organize and produce rhythmic, coordinated output from flexor-extensor motor pools that manifests as intra- and interlimb movements defining locomotion (7). These circuits can be activated in vitro by application of neurotransmitters [*N*-methyl-D-aspartate (NMDA), serotonin (5-HT), and dopamine (the latter required for locomotor circuit activation in P3 to P5)] to produce a correlate of in vivo locomotion, called fictive locomotion (36), in which rhythmic bursts with an organized flexor-extensor and left-right pattern are produced. In P1 to P5 littermate controls during fictive locomotion, stable rhythmic alternation among bilateral flexor-related (rostral lumbar segments, L2), bilateral extensor-related (caudal lumbar segments, L5), and ipsilateral flexor-extensor-related ventral roots was recorded (Fig. 3E, representative of bursting observed throughout P1 to P5). In the first 24 hours after birth, apart from select instances where the normally alternating flexor-extensor activity drifted to synchrony (Fig. 3F, vertical line), spinal motor output in spinal *Tor1a* d-cko mice was largely similar to that of littermate controls (fig. S3). However, by P2, the previously normal alternating flexor-extensor bursting activity became disorganized (Fig. 3G and fig. S3), with prolonged bursting at the caudal extensor-related lumbar motor pools, variable burst durations in the rostral flexor-related motor pools, and coactivation between the flexor- and extensor-related spinal motor pools. By P4, ENG bursting was profoundly altered across the lumbar spinal cord (Fig. 3H).

Neural oscillations are defined by their power, frequency, and phase relationship over time. To determine how the spinal *Tor1a* d-cko fundamentally alters neural output, we used tiered wavelet transformations (38). We first isolated the dominant power frequency bands and cycle durations at individual roots (fig. S3, A to E). We then proceeded with a set of cross-root wavelet transformations to extract the shared power, burst frequency, cycle duration, and phase relationships that define (i) left-right extensor-related (bilateral caudal roots, L4 and L5), (ii) left-right flexor-related (bilateral rostral, L1 and L2), and (iii) ipsilateral flexor-extensor-related neural activity. The resultant cross-root convolutions were plotted (Fig. 3, I to M), and the dominant (high-power) cross-root burst frequency, cycle duration (Fig. S3), and phase relationships over time were extracted for quantitative analysis of spinal motor output.

Littermate controls showed a consistent dominant high-power frequency band confined to 0.125 to 0.50 Hz for each root pair assessed (Fig. 3, I and M). This power frequency profile was also observed in spinal *Tor1a* d-cko mice at P1 (Fig. 3J). However, by P3 (Fig. 3K), spinal *Tor1a* d-cko mice showed a disruption to the power-frequency spectrum with a downward shift in burst frequency such that by P5, the dominant power frequency band was ~0.016 Hz (Fig. 3L). Extracting the shared frequencies from the high-power bands revealed a decrease in drug-induced burst frequency in all root pairs assessed (Fig. 3, N to P). This decrease in burst frequency translated to a ~four- to fivefold increase in the cross-root burst cycle duration (fig. S3, F to H).

After establishing the *Tor1a* conditional knockout-induced changes to the power frequency profile, we shifted our focus to cross-root burst coordination, a correlate of the disorganized movements that affect people with dystonia. Cross-root burst

coordination data were extracted from the dominant power frequency bands and plotted on circular graphs, wherein 0° denotes in-phase synchrony and 180° reflects out-of-phase alternation.

The classic locomotor profile of out-of-phase bursting activity among bilateral extensors, bilateral flexors, and ipsilateral flexor-extensors (7) was observed in the littermate controls, with phase data concentrated at 180° (Fig. 3, Q to S). For the most part, the burst coordination observed in P1 spinal *Tor1a* d-cko mice was broadly similar to that seen in littermate controls (fig. S3L). However, this normal bursting profile became disrupted at P2 to P5, wherein there was a predominant shift in the bursting activity toward in-phase synchrony (Fig. 3, Q to S). Cross-root coherence remained above 0.8 for all root pairs examined (fig. S3, I to K), suggesting that disruption to rhythmic bursting observed in one root was largely related or predictive of the disrupted bursting activity observed in the other root. Together, these data reveal that *Tor1a*-deleted spinal circuits directly produce excessive spontaneous activity at rest and disorganized motor output during locomotion.

Spinal monosynaptic reflexes are impaired in spinal *Tor1a* conditional knockouts

Given the spinal locomotor circuit dysfunction, we next looked at the most basic spinal circuit, one that can also be readily studied in humans: the monosynaptic (myotatic) reflex. Case reports indicate that individuals with generalized dystonia, including genetically confirmed *DYT1-TOR1A*, show diminished monosynaptic reflex amplitudes (12, 39), increased variability in the evoked response amplitude (12, 13), and the infiltration of aberrant asynchronous activity (12). We thus systematically assessed the monosynaptic reflex across the lumbar roots in spinal *Tor1a* d-cko mice (L1 to L5) at an age range where the dystonic phenotype was fully penetrant in the hindlimbs (P7 to P13).

Graded stimuli of increasing intensity were applied to dorsal roots, and the evoked monosynaptic reflexes were recorded from ventral roots (Fig. 4A, bottom). Plotting representative monosynaptic reflexes revealed a spatiotemporal pattern parallel to the dystonic-like phenotype. Compared with age-matched littermate controls, spinal reflexes in *Tor1a* d-cko mice were abnormal in the caudal-most root at P7, but the triphasic waveform appeared normal in the rostral roots (L1 and L3) (Fig. 4B). With increasing postnatal age, the impairments to the monosynaptic spinal reflex spread rostrally, affecting L3 and L1 by P11 to P13. Examination of the constituent components of the reflex responses (Fig. 4C) revealed that the reflexes in spinal-restricted *Tor1a* d-cko mice had lower amplitudes (fig. S4F) and longer durations (Fig. 4, D to F, and fig. S4G), with multiple asynchronous peaks (fig. S4, A to E). In addition to these impairments in the reflex waveforms, the latency to onset, a measure largely dependent on afferent conduction, was increased in spinal *Tor1a* d-cko mice compared with littermate controls in all segments at all ages (Fig. 4, G to I, and fig. S4H). Together, these data suggest that there is a caudal-to-rostral progression in monosynaptic spinal reflex impairments during postnatal maturation, including a dispersion of the reflex across time.

Spinal *Tor1a* deletion leads to distributed pathophysiology in the monosynaptic reflex

We next sought to gain mechanistic insights into reflex dysfunction by interrogating the four constituent components of this reflex arc: (i) proprioceptive afferents in the dorsal roots, (ii) synapses with

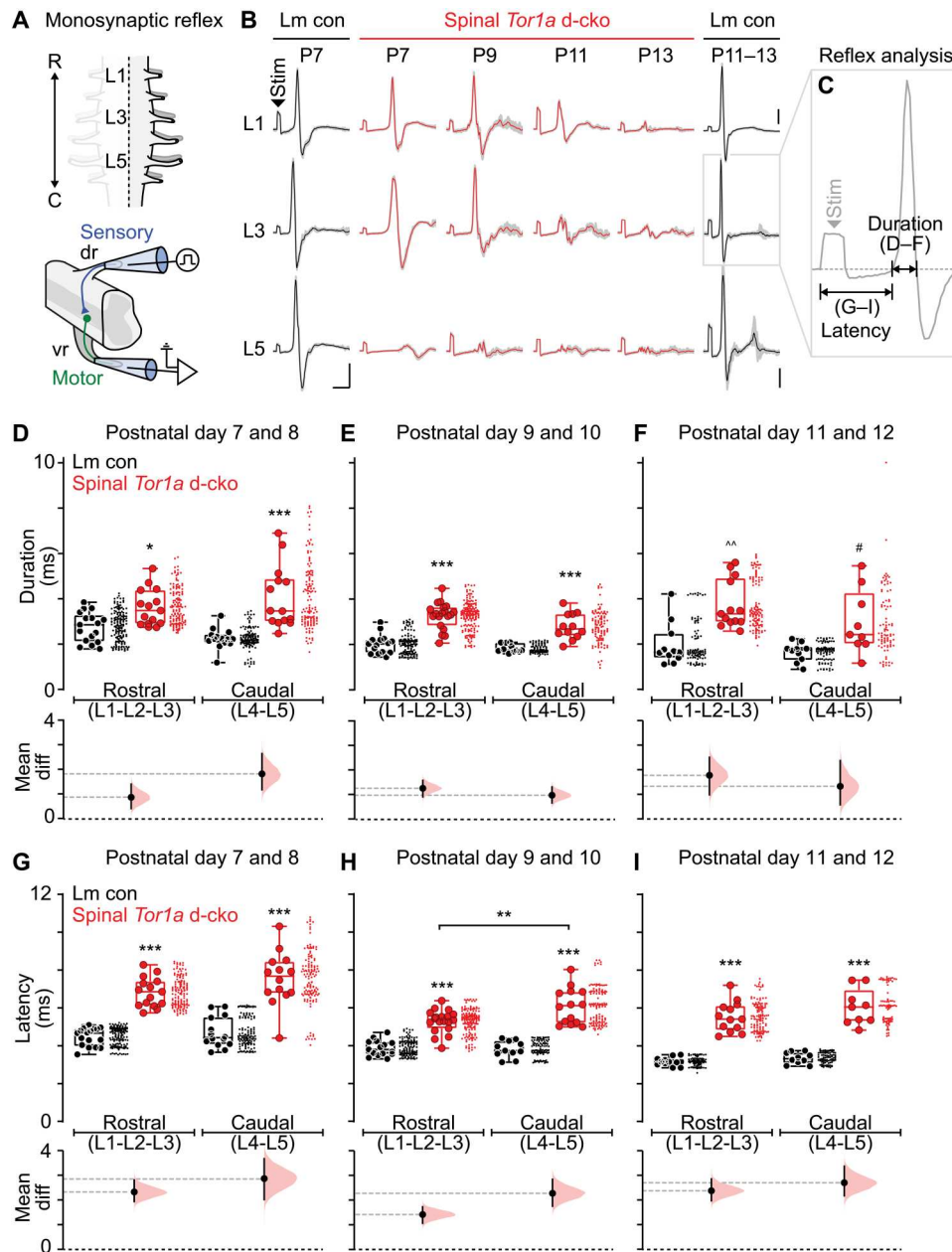


Fig. 4. Spinal-restricted *Tor1a* deletion impairs the monosynaptic reflex. (A) Schematic of the experimental design for recordings of the monosynaptic reflex (P7 to P13). R, rostral; C, caudal; dr, dorsal root; vr, ventral root. (B) Representative monosynaptic reflexes at 2.0x threshold. Data shown are average (bold) overlaid onto $N = 10$ sweeps (gray). Scale bars, $x = 5$ ms and $y = 0.05$ mV. (C) Quantification of monosynaptic reflex outcome measures: (D to F) response duration and (G to I) latency to onset in spinal *Tor1a* d-cko ($n = 21$) versus control ($n = 24$) mice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$, two-way analysis of variance (ANOVA) and Tukey's post hoc t test; ^^ $P < 0.001$, Mann-Whitney U test; # $P < 0.05$, independent t test with Welch's correction. Group data are shown (box plots) with individual means overlaid (circles) and mean differences (estimation plots). Dots, raw data, all reflexes analyzed per animal. [Also see table S1 and fig. S4.]

motor neurons, (iii) the motor neurons themselves, and (iv) efferent transmission in the ventral root. We focused on the caudal lumbar motor pools (L4 and L5), because they are the earliest affected. We used a ventral horn-ablated preparation (Fig. 5A) to determine whether motor neurons were intrinsically affected by the spinal-restricted *Tor1a* deletion. Motor neurons in spinal *Tor1a* d-cko mice appeared smaller than those in littermate controls (Fig. 5B). Although motor neurons in spinal *Tor1a* d-cko mice had similar

resting membrane potentials as control motor neurons (Fig. 5C), there was a reduction in whole-cell capacitance (Fig. 5D and fig. S5A) and a ~350% increase in input resistance (Fig. 5E and fig. S5B), consistent with the smaller cell size. Together, these data indicate that lumbar motor neurons are directly affected by *Tor1a* deletion.

However, smaller motor neurons alone could not explain all the changes in the monosynaptic reflex, so we next focused on the

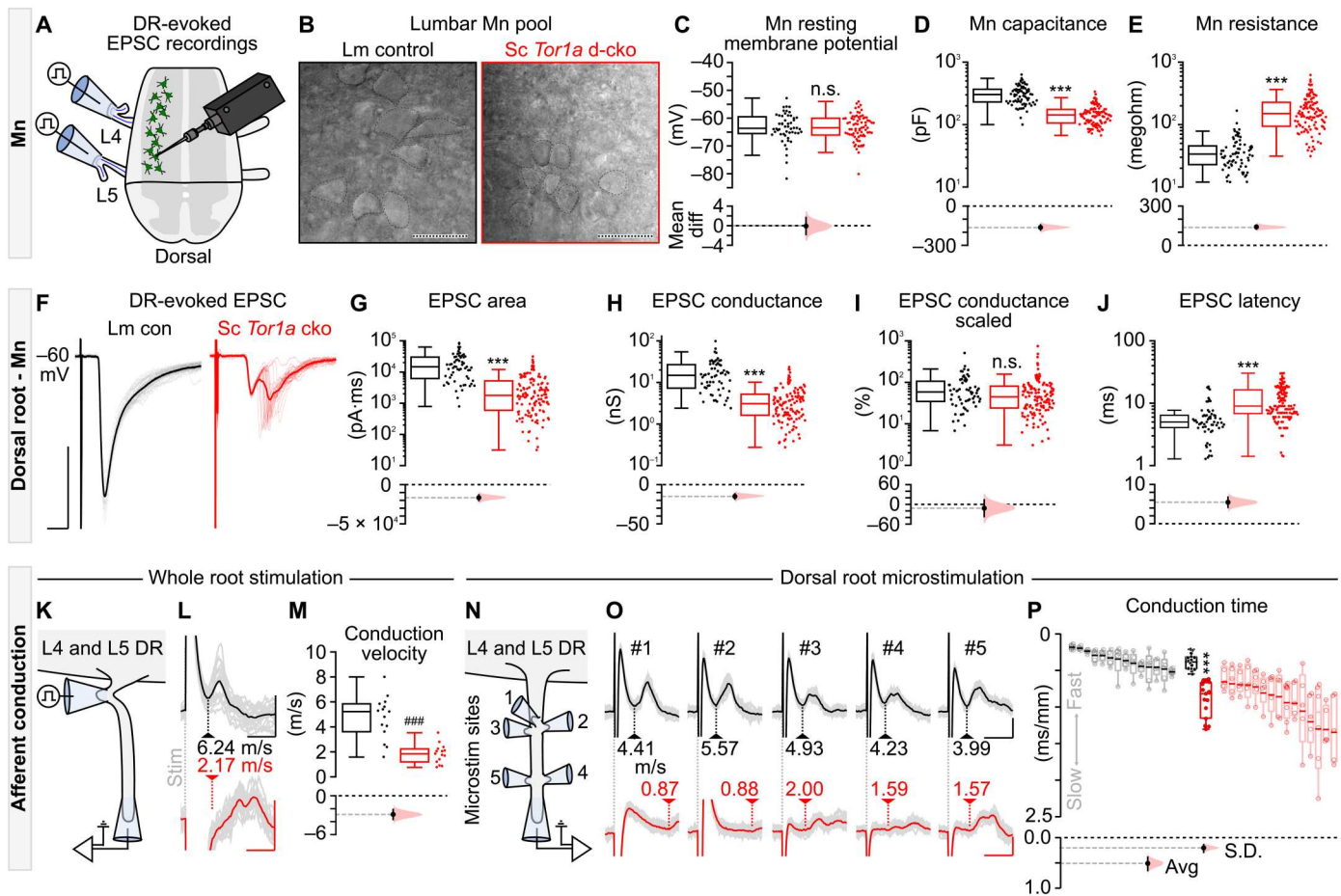


Fig. 5. All components of the monosynaptic reflex are impaired in spinal *Tor1a* d-cko mice. (A) Illustration of the experimental design to record afferent- and efferent-evoked excitatory postsynaptic potentials (EPSCs). Mn, motor neuron. (B) Differential interference contrast images of P9 motor neurons (scale bars, 50 μ m). Quantification of intrinsic properties (P1 to P13 n = no. of motor neurons): (C) resting membrane potential (n = 52 versus n = 77; P = 0.68, Mann-Whitney U test), (D) whole-cell capacitance (n = 73 versus n = 108; *** P < 0.0001, Mann-Whitney U test), and (E) input resistance (n = 73 versus n = 111). (F) Representative example, P7 dorsal root (DR)-evoked EPSC. Scale bars, x = 5 ms and y = 500 pA. Quantification of DR-evoked EPSCs: (G) area (n = 61 versus n = 130), (H) absolute conductance (n = 61 versus n = 128), (I) scaled conductance (n = 61 versus n = 126, P = 0.06), and (J) latency (n = 60 versus n = 131) (P1 to P13). Group data are shown (box plots) with mean differences (estimation plots). Dots, raw data, all responses analyzed per animal. (K) DR stimulation to estimate afferent conduction velocity. (L) Representative afferent volleys after whole root stimulation at threshold. Scale bars, x = 0.5 ms and y = 0.05 (black) or 0.025 mV (red) (L5 DR in P8 controls and P6 spinal *Tor1a* d-cko mice). (M) Quantification of L4 and L5 DR conduction velocity (P6 to P10, n = 15 versus n = 15; *** P < 0.0001; independent t test). (N and O) Representative examples of DR microstimulation afferent conduction velocities in control (black; scale bars, x = 0.5 ms and y = 0.05 mV) and spinal *Tor1a* d-cko mice (red; scale bars, x = 3 ms and y = 0.025 mV). (P) Quantification of afferent conduction time (P6 to P10, n = 14 versus n = 15 mice, average, *** P < 0.0001) and variance (SD, *** P < 0.0001). Averaged data are shown in black and red boxplots with individual means (within each root) overlaid (filled circles) and mean differences (estimation plots). The responses from individual roots, including raw (open circles) and averaged (bold line) values, are shown as desaturated boxplots adjacent to the averaged dataset. [Also see table S1 and fig. S5.]

afferent limb of the reflex using low-threshold stimulation of the dorsal roots. Compared with littermate controls, afferent-evoked excitatory postsynaptic currents (EPSCs) in spinal *Tor1a* d-cko mice had reduced amplitude, prolonged duration, and multiple asynchronous peaks (Fig. 5F), outcomes that were corroborated when we activated a subset of afferent fibers via discrete microstimulations (fig. S5, E to G). At all ages assessed, the EPSC area, a measure of charge carried, was decreased in motor neurons in spinal *Tor1a* d-cko mice compared with controls (Fig. 5G and fig. S5C). There was a decrease in EPSC conductance (Fig. 5H), but when scaled to input conductance, there was no difference (Fig. 5I), suggesting that the monosynaptic effects of afferent inputs to motor neurons are similar for littermate control and

spinal *Tor1a* d-cko mice. Of note, the total number of boutons expressing vesicular glutamate transporter 1 (vGluT1; a marker for primary afferents) in the ventral horn was reduced (2470 ± 30 versus 8280 ± 560 per hemisection; n = 2 controls and n = 3 spinal *Tor1a* d-cko mice at P18), as was the number in close apposition to motor neurons (11 ± 2 versus 25 ± 5 per motor neuron). At all ages tested, there was an increase in the latency to dorsal root-evoked monosynaptic EPSCs in spinal *Tor1a* d-cko mice as compared with controls (Fig. 5J and fig. S5D), a finding that parallels the increased latencies observed in extracellular recordings.

Although the longer latencies could result from impairments at synapses between group Ia afferents and motor neurons, they could also simply be due to deficits in afferent conduction itself. Thus, we

recorded L4 and L5 dorsal root volleys in response to root stimulation (Fig. 5K) and found slower afferent conduction velocities in spinal *Tor1a* d-cko mice as compared with controls (Fig. 5, L and M), suggesting that the longer latencies to EPSCs resulted from slower conduction velocities. However, increased latencies alone cannot account for the asynchronous peaks observed in EPSCs (fig. S5F). To this end, we microstimulated the dorsal roots at various sites, activating small subsets of fibers while recording from the distal root (Fig. 5, N and O). After scaling the conduction time by distance, we discovered that spinal *Tor1a* d-cko mice showed longer and variable conduction times as compared with controls (Fig. 5P), suggesting that the multiple peaks in the EPSCs resulted from time dispersion of the incoming afferent action potentials (fig. S5, F and G). That is, two effects occur in the dorsal roots of the spinal *Tor1a* d-cko mice: slower conduction velocities and increased variance of these velocities across fibers.

Given the conduction impairments in dorsal roots, we turned to the ventral roots to determine whether motor axons are also affected (fig. S5E). We found a decrease in efferent conduction velocity in spinal *Tor1a* d-cko mice as compared with controls (fig. S5, H to J). Responses to microstimulation also revealed increased scaled conduction times and variances in the ventral roots (fig. S5, K and L). In summary, all compartments of the monosynaptic reflex arc, from action potential conduction of sensory afferents to motor neurons to efferent output in the motor roots themselves, are vulnerable to *Tor1a* dysfunction and contribute to impaired sensory-motor integration in *Tor1a* d-cko mice.

DISCUSSION

Our results suggest that spinal circuit dysfunction is a key contributor to the pathophysiology of DYT1-*TOR1A* dystonia. By confining *Tor1a* deletion to the spinal cord and DRG neurons while leaving normal expression in the brain, mice phenotypically express a generalized torsional dystonia, have an ultrastructural signature indicating loss of function of torsinA (with unknown relevance to pathophysiology) in spinal but not brain neurons, have spinal locomotor circuit dysfunction, and have abnormal monosynaptic sensorimotor reflexes (27, 30, 40).

Coexisting with the motor impairments were signs of sensory dysfunction, with increased variance in the conduction velocities of the fastest dorsal root fibers in the *Tor1a* d-cko mice. Although not a major feature of human DYT1-*TOR1A* dystonia, sensory abnormalities have been reported (41), and sensory tricks, or gestes antagonistes, can help to alleviate the symptoms and signs of some dystonias (42). Given that *Cdx2::FlpO* directs recombinase activity to both spinal and DRG neurons (25), it is reasonable to ask whether the sensory dysfunction observed in spinal *Tor1a* d-cko mice may be due, in part, to the conditional knockout of *Tor1a* in DRG sensory neurons. That said, few DRG neurons showed NE malformations. Confining the *Tor1a* conditional knockout to DRG sensory neurons does not produce early-onset generalized torsional dystonia. Together, these data implicate spinal circuits and not primary afferents as the key substrate for dystonia pathophysiology in the spinal *Tor1a* d-cko model.

Our study has limitations. We are reporting a biallelic knockout of *Tor1a*, and human DYT1-*TOR1A* dystonia is largely associated with a mutation in a single allele. Moreover, we were not able to test whether DBS, one of the most effective treatment options for

DYT1-*TOR1A*, could alleviate dystonic pathophysiology in the spinal *Tor1a* d-cko model. Testing DBS in spinal *Tor1a* d-cko mice is not technically feasible because of the combination of rapid onset and progression of motor signs in preweaned, undersized pups, the size of the necessary hardware, and the expected duration of stimulation needed for alleviation of dystonic signs.

We performed a biallelic knockout of *Tor1a* to unambiguously test our hypothesis that dysfunctional spinal circuits could lead to dystonic pathophysiology; that is, we aimed to produce a phenotypically penetrant mouse model. Whereas children with biallelic *TOR1A* mutations have been identified with increasing frequency (43, 44), most adults with DYT1-*TOR1A* have a single-allele mutation that is associated with reduced penetrance (~30%) and variable phenotypic expression (45). Conversely, the spinal *Tor1a* d-cko mouse model shows complete penetrance: 100% of all the genotype-confirmed biallelic knockouts develop early-onset generalized torsional dystonia, suggesting that this model may reflect a more fully penetrant form of the human heterozygote condition. Humans with biallelic *TOR1A* mutations have arthrogryposis multiplex congenita 5, which usually includes flexor contractures that present predominantly prenatally or very early postnatally, as well as kyphosis/scoliosis (44). These deformities may be secondary to abnormal neuromuscular activity (46) and hence could potentially result from severe dystonia-related motor neuron activity during fetal development. Notwithstanding, the spinal *Tor1a* d-cko model is similar to the human monoallelic condition with respect to phenotype and physiology.

Phenotypically, motor signs in the spinal *Tor1a* d-cko mice parallel severe human DYT1-*TOR1A*: Dysfunction emerges early in life in a lower extremity and then spreads in a caudo-rostral fashion during developmental maturation until becoming fixed below the head. Thus far, previous rodent models have not reported or recapitulated this pathognomonic feature of DYT1-*TOR1A*, including conditional-ready models wherein *Tor1a* is manipulated in the cortex (34), basal forebrain (47, 48), striatum (28), or cerebellum (49), key nodes in prevailing models of dystonia (15). Furthermore, in spinal *Tor1a* d-cko mice, the phenotypic signatures of DYT1-*TOR1A* dystonia (abnormal posturing, truncal torsion, and intermittent tremulousness) manifest during naturalistic behavior when the pups are resting or moving about the environment.

Physiologically, we have shown that spinal *Tor1a* d-cko mice bear the three primary pathophysiological signatures of DYT1-*TOR1A* dystonia: (i) spontaneous muscle contractions at rest (10), (ii) excessive, sustained contractions during voluntary movements (10, 11), and (iii) altered sensory-motor reflexes (12, 13). To date, there has been limited study of the pathophysiological mechanisms underlying these signatures. Equipped with a fully penetrant mouse model that consistently and reproducibly develops dystonia and a suite of spinal cord preparations to probe sensory-motor dysfunction, we systematically interrogated the precipitating pathophysiological changes of early-onset generalized torsional dystonia. Recordings from isolated hindlimb motor neuron pools revealed that excessive spontaneous muscle contractions, including coactivation of motor antagonists, can be directly produced by dysfunctional spinal circuits. Much like the *in vivo* phenotype, there are caudo-rostral generalizations in spinal circuit dysfunction over postnatal development.

The spinal cord is composed of neural circuits that control the basic syllables of movement, including reciprocal inhibition to

change a joint angle, coexcitation of flexor and extensor motor neurons to stabilize a joint, and coinhibition of these motor neurons to allow the joint to move freely in biomechanical space (50). These syllables are concatenated across time to form functional movement (23). In dystonia, there is abnormal control of these fundamental syllables akin to a paraphasia of movement. Thus, we pursued the logic that spinal circuit dysfunction could lead to the signs of DYT1-TOR1A.

There have been some previous data pointing to spinal circuit dysfunction in dystonia. *Dyt1-Tor1a* animal studies have shown NE malformations in spinal neurons (27, 30), spinal motor neuron loss (28), and reduced spinal GABAergic inputs to primary afferent fibers (51). In non-Dyt1 dystonia models, *Lamb1t* mice have coincident EMG activity between opposing muscles, a phenomenon that persists after spinal transection and thus directly implicates dysfunctional spinal circuits (52). In addition, in people affected by DYT1-TOR1A, analyses of spinal reflexes indicate that they may have impairments in monosynaptic stretch reflexes (12, 13) and reciprocal inhibition (10, 53, 54). Although these reflexes are mediated by spinal circuits, the impairments observed have been attributed to dysfunction of descending systems (53). However, spinal circuits are complex and form specialized, multilayered networks that integrate supraspinal, spinal, and sensory inputs to organize motor output (55). Thus, in dystonia pathophysiology, it is logical to consider spinal circuits as a critical nexus for neurological dysfunction and movement disorganization in dystonia.

We have shown that spinal circuit dysfunction can recapitulate one of the most severe forms of primary dystonia. That is, in the homozygous condition, descending command signals cannot override or compensate for spinal circuit dysfunction such that generalized torsional dystonia manifests over postnatal time. However, one of the most effective treatment options for DYT1-TOR1A is DBS of a site in the basal ganglia, the globus pallidus internus (56). If spinal circuit dysfunction leads to disorganized movements, then why is DBS an effective treatment for dystonia?

Compared with other DBS-treated movement disorders such as essential tremor or Parkinson's disease wherein stimulation offers rapid symptom relief within seconds to hours (57), many weeks to months of continuous stimulation are typically required before tonic dystonic movements show improvement (56). [This progressive improvement over months is also seen after pallidotomy (58).] This delay to symptom amelioration could result from neuroplastic mechanisms: a long-term process with adaptive effects that can be localized or distributed via interconnected circuits (57). Maladaptive neuroplasticity is a widely recognized contributing factor to dystonia (59), with miswired circuitry implicated in the local motor planning ensemble (basal ganglia loops) (60) and distant yet connected circuits such as the corticospinal tract (60). If dystonia-producing maladaptive plasticity is spatiotemporally distributed across remote yet interconnected circuits (including spinal circuits, as shown here), then dystonia-alleviating adaptive plasticity is likely similarly secured. Evidence for spinal plasticity has been shown: Long-term DBS gradually improved spinal-mediated reciprocal inhibition, restoring agonist-antagonist coordination in the forearms of individuals with generalized dystonia (61). That is, it seems likely that DBS-mediated improvement of dystonia involves adaptive plasticity throughout interconnected motor ensembles, including spinal circuits.

That is, that spinal cord circuits have the capacity to adapt is not a new thought (62). Findings over decades of spinal cord injury research have established that spinal circuits directly produce organized movements and are intrinsically capable of mediating functional recovery. For example, classic experiments in cats established that after a complete spinal cord transection and resultant paralysis, several weeks of activity-based training can lead to isolated spinal circuits, devoid of descending inputs, to develop the capacity to produce full body weight-supported stepping (63, 64). These fundamental studies have been clinically translated: Chronic lumbosacral epidural stimulation, paired with activity-based training, can restore function in paralyzed humans (65).

To conclude, with this model of DYT1-TOR1A dystonia, we have a newfound entry point into investigating the complex pathophysiology of the disease. As a circuitopathy, dystonia can be considered as a process that affects motor circuits throughout the central nervous system, including those in the spinal cord. The notion that spinal motor circuits are simple relays between the brain and muscles has long been dispelled. However, spinal circuit dysfunction is rarely considered in movement disorder pathophysiology. We would suggest that new treatment strategies for DYT1-TOR1A dystonia could be aimed at addressing the pathophysiology underlying symptoms, the circuits of which are largely resident in the spinal cord.

MATERIALS AND METHODS

Study design

The goal of this study was to determine to what extent spinal circuit dysfunction contributes to the pathophysiology of early-onset generalized torsional dystonia in mice. Our objectives were to develop and validate a spinal-restricted conditional knockout of *Tor1a*, determine whether spinal circuit dysfunction leads to early-onset generalized dystonia, and quantify the extent to which spinal circuit dysfunction contributes to the pathophysiology of this movement disorder.

Experiments were performed in preweaned mice with the date of birth recorded as P0. Mice were arbitrarily allocated to the various sets of experiments (qPCR, EMG, and ENG) and arbitrarily selected from the litter on a day-by-day basis, randomizing the age of allocation. Experiments were performed blinded to genotype. When blinding was impossible (overt genotype-phenotype), data were collected and coded for subsequent blinded analysis. Because of the nature of this work, a priori power analyses were not feasible. Sample sizes were estimated on the basis of our previous experience with each technique. Outliers are shown in the raw data and reported in figure legends. Any data that were excluded from this study are described in the relevant sections.

Animal procedures were approved by the University College London Animal Welfare and Ethical Review Body and carried out in accordance with the Animal (Scientific Procedures) Act 1986 (Home Office, UK) under project license 70/9098, with experiment metadata reported following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3R). *Tor1a*-frt mice were generated by Cyagen Biosciences and were made available through the Mutant Mouse Resource and Research Center (no. 69706). Additional mice were obtained from the following sources: *Cdx2::FlpO* (M. Goulding,

Salk Institute, USA), Advillin-cre (JAX no. 032536), and Tor1a-flox (JAX no. 025832).

qPCR

P18 mice ($n = 14$) were anesthetized, and organs were harvested for qPCR using probes for *Tor1a*, *Actb*, *Gapdh*, and *Hprt*. *Tor1a* fold expression values were estimated using $\Delta\Delta C_t$ method with *Hprt* or *Actb* serving as housekeeping gene.

The same methods were used for quantifying *Tor1a* expression in $n = 6$ P58 and P59 littermate controls (Avil^{wt/wt};Tor1a^{wt/flox}) and $n = 5$ DRG *Tor1a* d-cko mice (Avil^{wt/cre};Tor1a^{flox/flox}). Two outliers, one from each group, were excluded from analysis (tissue contamination).

Western blots

P18 mice ($n = 7$) were anesthetized, and organs were harvested for Western blot analysis using rabbit anti-torsinA (1:1000) followed by goat anti-rabbit horseradish peroxidase (HRP)-conjugated species anti-rabbit secondary antibody (1:1000). Bands were detected using HRP substrate and imaged for subsequent analysis.

Ultrastructure

P18 ($n = 8$) mice were anesthetized and transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Tissue was harvested and processed for ultrastructure analysis.

Immunohistochemistry

P18 ($n = 5$) mice were anesthetized and transcardially perfused with 0.1 M PBS followed by 4% PFA. Spinal cords were dissected, cryo-protected, embedded, cross-sectioned, and stained with goat anti-choline acetyltransferase (1:250) and guinea pig anti-vGluT1 (1:2000) followed by donkey anti-goat Alexa Fluor 488 (1:1000) and donkey anti-guinea pig Alexa Fluor 647 (1:1000). Images were acquired and analyzed using custom-written scripts.

Behavior

Mice ($n = 14$) were videotaped (60 to 90 s) starting at P1 to P3 and continuing every other day until P14 and P15. Five external raters experienced with mouse behavior were then selected to provide unbiased phenotypic scoring.

In vivo electrophysiology

P17 ($n = 2$) and P19 ($n = 4$) spinal *Tor1a* d-cko mice as well as $N = 4$ P18 C57Bl/6J mice were anesthetized, and recording electrodes were inserted into the gastrocnemius and tibialis anterior muscles. Recordings were performed with signals amplified, bandpass-filtered, digitized, and saved for offline analysis using custom-written scripts.

In vitro electrophysiology: Spontaneous activity and drug-induced fictive locomotion

Whole spinal cords were isolated from $n = 5$ P1 to P4 littermate controls and $n = 11$ P1 to P5 spinal *Tor1a* d-cko pups and transferred to recording chambers perfused with artificial cerebrospinal fluid (aCSF) that was continuously bubbled with 95% O₂/5% CO₂. Recording electrodes were attached to rostral (bilateral L1 or L2) and caudal (bilateral L4 or L5) lumbar ventral roots. Signals were differentially amplified, bandpass-filtered, digitized, and saved for

offline analysis of spontaneous activity and drug-induced fictive locomotion.

In vitro electrophysiology: Monosynaptic reflex

Whole spinal cords were isolated and hemisected from $n = 24$ littermate controls and $n = 21$ spinal *Tor1a* d-cko pups aged P7 to P13. Spinal cords were transferred to recording chambers perfused with aCSF (bubbled with 95% O₂/5% CO₂). Suction electrodes connected to stimulus isolators were attached to the L1 to L5 dorsal roots. L1 to L5 ventral roots were attached to extracellular recording electrodes connected to custom-made bipolar electrode holders and headstage amplifier setup. Motor threshold was identified for each root, after which $N = 10$ sweeps/trials/roots were recorded at multiples of threshold. Signals were differentially amplified, bandpass-filtered, digitized, and saved for offline analysis.

In vitro electrophysiology: Patch-clamp recordings and conduction velocity estimates

Spinal cords were isolated from $n = 11$ littermate controls and $n = 16$ spinal *Tor1a* d-cko mice aged P1 to P13. Tissue was transferred to a recording chamber perfused with aCSF (bubbled with 95% O₂/5% CO₂). Glass pipettes were pulled, fire-polished to a resistance of ~2 to 4 megohms, and filled with an intracellular solution. EPSCs were recorded after dorsal root stimulation. Root potentials were recorded after targeted stimulation of different sites along the spinal root. Distances between stimulation and recording sites were recorded to estimate conduction velocities.

Statistical analysis

Statistical analyses were performed using OriginLab, Microsoft Excel, Data Analysis with Bootstrap-Coupled ESTimation Python script, MATLAB, MATLAB-based SpinalCoreN, GraphPad Prism, and SigmaPlot. Differences between groups were considered statistically significant at $P \leq 0.05$. Two-tailed P values are reported. Outliers (values exceeding average ± 3 SD) were excluded from analysis. Descriptive statistics are reported in table S1.

Supplementary Materials

This PDF file includes:

Supplementary Materials and Methods

Fig. S1 to S5

Table S1

References (66–74)

Other Supplementary Material for this manuscript includes the following:

Data file S1

Movies S1 to S7

MDAR Reproducibility Checklist

[View/request a protocol for this paper from Bio-protocol.](#)

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