The precision of skilled movement depends on sensory feedback and its refinement by local inhibitory microcircuits. One specialized set of spinal GABAergic interneurons forms axo–axonic contacts with the central terminals of sensory afferents, exerting presynaptic inhibitory control over sensory–motor transmission. The inability to achieve selective access to the GABAergic neurons responsible for this unorthodox inhibitory mechanism has left unresolved the contribution of presynaptic inhibition to motor behaviour. We used Gad2 as a genetic entry point to manipulate the interneurons that contact sensory terminals, and show that activation of these interneurons in mice elicits the defining physiological characteristics of presynaptic inhibition. Selective genetic ablation of Gad2–expressing interneurons severely perturbs goal–directed reaching movements, uncovering a pronounced and stereotypic forelimb motor oscillation, the core features of which are captured by modelling the consequences of sensory feedback at high gain. Our findings define the neural substrate of a genetically hardwired gain control system crucial for the smooth execution of movement.

Animals execute skilled limb movements with seemingly effortless precision, belying an elaborate series of neural transformations that direct each motor act. Spinal motor output relies on local inhibitory interneurons that shape the response of motor neurons to diverse excitatory inputs. Most inhibitory interneurons form direct postsynaptic connections with motor or premotor neurons, but a small subset of GABAergic interneurons instead forms axo–axonic contacts with sensory afferent terminals, regulating sensory–motor drive through a process of presynaptic inhibition (Fig. 1a). This presynaptic inhibitory system can be recruited by sensory, descending and local neuronal inputs, implying its pivotal role in the control of motor output. However, despite the occurrence of axo–axonic contacts at most sensory terminals, the predominance of postsynaptic inhibition has left unresolved the motor behavioural significance of this presynaptic control system.

Presynaptic inhibition has been characterized most extensively at proprioceptive sensory–motor synapses. Proprioceptors convey the state of muscle contraction to motor neurons, through direct and indirect feedback pathways. Elimination of proprioceptive feedback impairs
motor coordination\textsuperscript{10}, establishing a basal requirement for sensory transmission in motor control. Conversely, limiting the gain of proprioceptive feedback has been proposed, on theoretical grounds, to be a critical determinant of motor stability\textsuperscript{11}. In principle, the divisive nature of pre-synaptic inhibition provides an effective means of controlling sensory gain\textsuperscript{3,12–14}, but without a way to manipulate the relevant set of inhibitory interneurons it has not been possible to resolve whether, or how, pre-synaptic inhibition contributes to motor behaviour.

The inhibitory interneurons that form axo–axonic contacts with sensory terminals differ from other spinal GABAergic neurons in that they alone express GAD2 (also known as GAD65), one of two GABA-synthetic enzymes (Fig. 1a–c)\textsuperscript{7,15,16}. We have used Gad2 as a genetic entry point for manipulating presynaptic inhibitory interneurons in mice and assessing their role in motor behaviour. Our findings indicate that Gad2-expressing interneurons mediate presynaptic inhibition at sensory–motor synapses, and that selective elimination of these interneurons elicits motor oscillations during goal-directed reaching. The essential features of this motor perturbation can be captured by a simple model in which high-gain proprioceptive feedback induces limb oscillation. Features of this motor perturbation can be captured by a simple model in which high-gain proprioceptive feedback induces limb oscillation.

**Targeting GABA\textsubscript{pre} neurons**

We first asked whether the GABAergic interneurons that contact sensory terminals (GABA\textsubscript{pre} neurons) can be defined by transgene reporter expression in a Gad2-IRES-Cre (Gad2\textsuperscript{Cre}) mouse line\textsuperscript{17}. To mark Gad2-expressing interneurons (Gad2\textsuperscript{Cre} neurons) we injected a recombinant adeno-associated viral vector encoding a Cre-recombinase-dependent (FLEX) channelrhodopsin (ChR2)–yellow fluorescent protein (YFP) fusion protein into the spinal cord of Gad2\textsuperscript{Cre} mice.

For physiological studies of presynaptic inhibition we targeted Gad2\textsuperscript{Cre} neurons in early postnatal lumbar spinal cord (Supplementary Note 1)\textsuperscript{18}. At this stage Gad2 is expressed both by GABA\textsubscript{pre} neurons as well as by GABA\textsubscript{post} neurons that contact motor neurons and premotor interneurons (Fig. 1a–c)\textsuperscript{17}. Injection of FLEX-ChR2-YFP at postnatal day (p) 0–3 into lumbar segments resulted, 14–21 days later, in broad YFP expression with dense axonal labelling in the vicinity of motor neurons (Fig. 1d). At this stage 36% of GABAergic terminals near motor neurons derive from GABA\textsubscript{pre} neurons, and 64% from GABA\textsubscript{post} neurons. We found that 85% of GABA\textsubscript{pre} and 57% of GABA\textsubscript{post} boutons in the ventral spinal cord expressed ChR2–YFP (Fig. 1b–f). Thus early postnatal FLEX-ChR2-YFP injection marks GABA\textsubscript{pre} and GABA\textsubscript{post} boutons at similar incidence.

For motor behavioural studies we targeted Gad2\textsuperscript{Cre} neurons in adult cervical spinal cord (Supplementary Note 1 and Extended Data Fig. 1). Cervical injection of FLEX-ChR2-YFP at ∼p78, with analysis 14–21 days later, revealed that 78% of GABA\textsubscript{pre} and ∼1% of GABA\textsubscript{post} boutons expressed YFP (Fig. 1b, c, g–i). Thus adult Gad2\textsuperscript{Cre} transduction marks GABA\textsubscript{pre} neurons in a near-selective manner.

**Gad2\textsuperscript{Cre}–mediated presynaptic inhibition**

We determined whether activation of Gad2\textsuperscript{Cre} interneurons elicits the two hallmarks of presynaptic inhibition: primary afferent depolarization and suppression of sensory neurotransmitter release\textsuperscript{4}.

We first examined whether photoactivation of ChR2-expressing Gad2\textsuperscript{Cre} neurons entrains neuronal spiking. Recordings from ChR2–YFP\textsuperscript{+} neurons in p9–14 lumbar spinal cord preparations from Gad2\textsuperscript{Cre} mice injected with FLEX-ChR2-YFP at p0–3 (Fig. 2a) revealed that photostimulation (473 nm, ∼10 mW) elicited action potentials that followed frequencies up to ∼50 Hz (Fig. 2b–d)\textsuperscript{20}. Thus targeted ChR2 expression confers optical control of Gad2\textsuperscript{Cre} neuronal spiking.

We then determined whether GABA release upon Gad2\textsuperscript{Cre} neuronal photoactivation depolarizes sensory neurons, eliciting primary afferent depolarization (PAD; Fig. 2e)\textsuperscript{21}. Single-pulse photoactivation of Gad2\textsuperscript{Cre} interneurons elicited PAD with an amplitude and time course similar to that induced by dorsal root stimulation (Fig. 2e, f). Antagonists of GABA-A receptors, but not glycine receptors, abolished Gad2\textsuperscript{Cre} neuronal spiking.

**Figure 2** | Gad2\textsuperscript{Cre} photoactivation elicits presynaptic inhibition. a, Recording from ChR2–YFP\textsuperscript{+} (blue outline) Gad2\textsuperscript{Cre} neurons (red). b–d, Photostimulation (λ) induced currents (b) and action potentials (c), whose frequency (f\textsubscript{AP}) followed light-pulse frequency (f\textsubscript{L}) up to ∼50 Hz (top, n = 3). Distribution of interspike intervals (ISI) for f\textsubscript{L} = 50 Hz (bottom, 19.9 ± 0.6 ms s.e.m., n = 500 trials). e, Dorsal root stimulation (L5) and recording (L4). f, L5-evoked (top, DR) and light-evoked (bottom, λ = 473 nm; n = 4) PAD. Arrows, antidromic spikes. g, Light-evoked PAD without (Ctrl) and with SR 95531 (Gzb, 2 μM) or strychnine (Str, 5 μM). Plots (f, g) show PAD amplitude. h, Isolating sensory input during Gad2\textsuperscript{Cre} photoactivation. i, Motor neuron identified using differential interference contrast (DIC) optics, Hb9GFP expression and Alexa\textsuperscript{555} fill. j, k, Monosynaptic sensory–EPSCs (j, 90 trials, 10 Hz, arrow indicates sensory stimulation); onset latencies (Gaussian fit, red) estimated using EPSC waveform derivative (k; top, mean; bottom, individual traces; EPSC onsets, red). Mean onset latency 2.87 ± 0.18 ms; c\textsubscript{onset} 0.018 ± 0.0022; n = 19. l, Sensory-EPSCs (0.1 Hz; mean, bold; raw, faint) without (EPSC, black) or with photoactivation (EPSC\textsubscript{p}, blue; fifteen 1 ms light pulses at 50 Hz; 45 ms delay). Inset, EPSC suppression; n = 9. Values and error bars, mean ± s.e.m., except I, mean ± s.d. See Extended Data Figs 2, 4 for additional quantification.
neuron-evoked PAD (Fig. 2g), establishing its GABAergic character22. However, PAD reflects the depolarization of cutaneous as well as proprioceptive afferents23, prompting us to ask whether Gad2Cre neuron activation depolarizes proprioceptive afferents. At reduced temperatures in vivo PAD evokes transmitter release from proprioceptor terminals, depolarizing motor neurons (Supplementary Note 2)21. Consistent with this, we found that Gad2Cre photoactivation in vitro at 24–26 °C elicited a GABA-A and AMPA receptor-dependent motor neuron depolarization (Extended Data Fig. 2). Activation of Gad2Cre neurons therefore elicits PAD at proprioceptor terminals.

To assess the impact of Gad2Cre neuronal activation on sensory–motor transmission we isolated sensory input to motor neurons. Stimulation of individual L3 to L5 dorsal roots elicited monosynaptic excitatory postsynaptic currents (EPSCs) in motor neurons (mean amplitude 1.2 ± 0.3 nA s.e.m.; coefficient of variation of onset (cvonset) < 0.02; n = 19; Fig. 2h–k and Extended Data Fig. 3)23. Photoactivation of Gad2Cre neurons elicited a frequency-dependent reduction in sensory-evoked EPSC amplitude that persisted for >800 ms (Fig. 2l, inset; mean ± 40%, range 19–53% reduction in EPSC amplitude; two-tailed paired t test, P < 10−4; n = 9; Extended Data Fig. 4). Thus synchronous activation of Gad2Cre neurons elicits a long-lasting suppression of sensory-evoked EPSCs (Supplementary Note 3).

At the neonatal stages used for physiological analysis Gad2Cre marks both GABApre and GABApost neurons. Nevertheless, three findings indicate that Gad2Cre suppression of sensory-evoked EPSCs reflects presynaptic inhibition. First, Gad2Cre-evoked inhibitory postsynaptic currents (Gad2Cre-IPSCs) persisted for only ∼30 ms (Fig. 3a–c and Extended Data Fig. 5a–c), whereas Gad2Cre EPSC suppression lasted long after Gad2Cre-IPSCs had decayed (Extended Data Figs 4a and 5a, b)4. Second, Gad2Cre EPSC suppression is exclusively GABAergic, whereas coexpression of glyoxime by most GABApost neurons underlies the fact that Gad2Cre-IPSCs are predominantly glycinergic (Fig. 3a–g)24,25. Third, Gad2Cre EPSC suppression did not alter EPSC waveforms, arguing against the idea that the reduction in EPSC amplitude reflects an increased motor neuron membrane conductance (Extended Data Fig. 5d–j). These findings reveal that GABApost neurons make only a minimal contribution to the inhibition of sensory-evoked EPSCs, thus implicating GABApre neurons.

The second core feature of presynaptic inhibition is a reduced probability of sensory transmitter release16,27. Modelling shows that this reduction can be captured by the kinetics of decay in amplitude of successive sensory-evoked EPSCs (Fig. 3h, i)28,29. We found that upon repetitive dorsal root stimulation, photoactivation of Gad2Cre neurons elicited a reduction in the rate of EPSC amplitude decay that was readily fit by a decrease in the release probability parameter of a short-term depression model (ρ2 = 0.69 ± 0.03, control; 0.50 ± 0.05 s.e.m., photoactivation; two-tailed paired t test, P < 0.001, n = 9; Fig. 3i, j and Extended Data Fig. 6)27. The fidelity of this fit indicates that activation of Gad2Cre neurons reduces sensory transmitter release probability. Therefore Gad2Cre neurons mediate both classical features of presynaptic inhibition at sensory–motor synapses.

Limb oscillation after GABApre ablation

In primates presynaptic inhibition has been implicated in the control of forelimb movement30, prompting us to use goal-directed reaching in mice as a behavioural routine for examining the impact of removing presynaptic inhibition (Supplementary Note 1). We eliminated GABApre neurons by injecting an adeno-associated virus encoding a Cre-recombinase-dependent diphtheria toxin receptor-green fluorescent protein fusion (FLEX-DTR-GFP) unilaterally into C3–T1 spinal segments in p56–84 Gad2Cre mice (Fig. 4a)31. Administration of diphtheria toxin (DT) 14–21 days later resulted in a >90% depletion of Gad2Cre/GAD1+ boutons on ventral vGluT1+ sensory terminals, without altering the number of GABApost Gad2CreOFF/GAD1+ boutons (Fig. 4b). Thus adult Gad2Cre-based DTR targeting achieves selective elimination of GABApre neurons. Mice lacking GABApre neurons exhibited two prominent behavioural phenotypes: impaired reaching and forelimb scratching.

To analyse forelimb motor behaviour, mice were trained to reach for and retrieve a food pellet located on the far side of a narrow access window32,33, with reaches analysed before and after GABApre neuronal ablation (Fig. 4c, d). DT administration elicited a marked degradation in reach accuracy, such that by the seventh post-DT day fewer than 5% of trials were successful, compared to a pre-DT success rate of ∼50% (Fig. 4e). We detected no change in left-right forelimb alternation, nor in the accuracy of forepaw placement in a horizontal ladder stepping task (Fig. 4f)31,32. Although these findings do not preclude a role for presynaptic inhibition in locomotor control, they do indicate a degree of task-selectivity in the degradation of motor performance.

To evaluate forelimb movement we quantified limb kinematics by tracking a reflective marker attached to the right forepaw (Fig. 4d)31, comparing reach trajectory and velocity in individual mice before and after GABApre ablation. Before DT administration mice displayed smooth paw trajectories that varied little across trials or with trial success (Extended Data Fig. 7a and Supplementary Videos 1 and 2)31. In contrast, after GABApre ablation the stereotypically smooth nature of reaches was lost and paw trajectories exhibited frequent reversals in direction (Fig. 5a–c, Figure 3 | GABApre photoactivation reduces sensory transmitter release. a, Distinguishing GABApre from GABApost inhibition. b–g, Pharmacological analysis. b–d, Light-evoked (1) IPSCs plus strychnine (Str; n = 5) (b), SR 95531 (Gbz; n = 3) and CGP 54626 (Gcp, 2.5 μM; n = 3) (c); IPSC index, ratio of IPSC amplitude with drug to control (d). e–g, Gad2Cre suppression of sensory-EPSCs (with photostimulation, blue; without, black) with strychnine (Str, n = 3) (e); SR 95531 and CGP 54626 (Gbz/Gcp, n = 4) (f); EPSC index, no effect = 1; drug abolishes EPSC-suppression = 0 (see Methods for description and Extended Data Fig. 5 for statistics) (g). h, Isolation of GABApre inhibitory action. i, Sensory-EPSCs (10 pulses, 25 Hz; mean, bold; raw, faint) without (black) or with photostimulation (blue, with black control superimposed for comparison). j, Normalized mean EPSC amplitudes from i. Grey, exponential fits to model-generated EPSC amplitudes. Values and error bars, mean ± s.e.m.
Figure 4 | Selective GABApre ablation. a, After C3−T1 FLEX-DTR-GFP injection into adult Gad2Cre mice, GABApre boutons (GAD2Cre, red) contact proprioceptor terminals (vGlut1Cre, blue) pre- but not post-DT administration. Only GAD1-marked GABApost boutons remain post-DT. vGlut1Creboutons with GABApre contacts, pre-DT 88 ± 2% (n = 2,058 boutons in 4 mice); post-DT 9 ± 1% (n = 4,347boutons in 3 mice). b, Experimental timeline. c, Reaching kinematics assay (see Methods). d, Stepping accuracy, horizontal ladder assay (n = 4). Values and error bars, mean ± s.e.m. See Extended Data Fig. 9 and Extended Data Table 1 for statistics.

Extended Data Fig. 7b, Extended Data Table 1 and Supplementary Videos 3 and 4). Kinematic defects were restricted to the ‘reach’ phase — before the paw passes through the access window (Fig. 5c and Extended Data Table 1). Kinematic defects during the late ‘grab’ phase — when the paw passes through the access window (Fig. 5c and Extended Data Table 1 and Supplementary Videos 7b, Extended Data Table 1 for statistics).

To assess the periodicity of aberrant forelimb movements upon GABApre ablation we examined the temporal structure of forepaw kinematics. The spectral power of pre-DT reaches decayed steadily with increasing oscillation frequency (Fig. 5g). In contrast, post-DT reach oscillations exhibited an ~7.4-fold increase in peak spectral amplitude within the 18–22 Hz frequency range, focused at 19.5 ± 0.5 Hz s.e.m., with little variability across reaches and animals (CVfrequency = 0.06, n = 5; Fig. 5g).

We also detected a sub-harmonic series with spectral peaks at 10.8 ± 0.5 Hz and 5.9 ± 0.3 Hz (Fig. 5f). The decay of post-DT oscillations could be fit by a single exponential function with a time constant of 77 ± 3 ms s.e.m., again consistent across animals (CVdecay = 0.08, n = 5; Fig. 5b, i). Thus elimination of GABApre neurons uncovers a structured oscillation of constant frequency and decay time, defining features of a damped harmonic oscillator.

A GABApre gain control system

Prior models of sensory–motor control have proposed that increasing the gain of proprioceptive feedback elicits motor oscillation 11. Can a simple model of sensory–motor feedback gain provide a theoretical framework for interpreting the oscillations that follow GABApre ablation? We simulated a simplified forelimb joint controlled by flexor and extensor sensory and subject to gain-modulated sensory feedback, fitting the frequency and decay time of the model’s single oscillatory eigenmode to experimentally-derived post-DT values (Fig. 6a, b, Extended Data Fig. 8a, b and Supplementary Note 4). At low feedback gain this model displayed smooth joint extension, resembling pre-DT reach velocity profiles (Fig. 6a, c; compare with Fig. 5d, top). At high gain, simulating the loss of divisive (presynaptic) inhibition, joint extension was dominated by a cycle of flexor-extensor alternation that resembled post-DT reach velocity profiles (Fig. 6b, d; compare with Fig. 5d, bottom). Reducing subtractive (postsynaptic) inhibition did not result in similar oscillations (Fig. 6c, green line, Supplementary Note 4 and Supplementary Discussion). This theoretical analysis suggests that oscillations produced by elimination of presynaptic inhibition cannot be prevented by remaining intact postsynaptic inhibition, consistent with our experimental observations. How do joint oscillations evolve as gain increases? Across a wide range of gain levels, the oscillatory frequency and decay times were constrained to a narrow set of values (Fig. 6e, f and Supplementary Note 4), providing a potential basis for the constancy of motor oscillation observed across mice. Moreover, similar oscillations emerged across multiple feedback delays (Extended Data Fig. 8c–h), accommodating the potential involvement of direct and indirect sensory feedback circuits (Supplementary Note 5). Thus a simple model of proprioceptive feedback-driven oscillation can account for the principal motor deficits that emerge upon elimination of presynaptic inhibition.
Finally, we considered whether this proprioceptive view of feedback-driven oscillation is challenged by the forelimb scratching behaviour observed after GABApre ablation (Extended Data Fig. 9a, b). In particular, we asked whether defects in forelimb reach could reflect enhanced cutaneous input. We applied the Na⁺ channel blocker lidocaine topically to the forelimb of GABApre-ablated mice, with the intent of selectively inactivating cutaneous sensory endings. Lidocaine eliminated scratching behaviour but left reaching defects and the ~20 Hz forelimb oscillation unaffected (Extended Data Fig. 9c–f and Supplementary Video 6). These results argue against the notion that enhanced cutaneous gain contributes to motor oscillation and aberrant reaching. Our findings therefore place GABApre neurons at the core of a proprioceptive gain control system for suppression of oscillations during goal-directed forelimb movement.

Discussion

Despite long appreciation of presynaptic inhibitory control, its contribution to motor behaviour has remained unclear. We have identified, activated and eliminated the spinal GABAergic interneurons responsible for presynaptic inhibition at sensory synapses, revealing their central role in suppressing motor oscillation during forelimb movement. Our findings address three core issues in sensory–motor control: the behavioural relevance of sensory gain scaling; the neural underpinnings of smooth limb trajectory; and the grain of neural circuitry that engages presynaptic inhibition.

The rich repertoire of mammalian limb movements demands that proprioceptive afferents sustain a wide range of firing frequencies as they strive to supply task-appropriate feedback. The dynamic character of sensory firing implies in principle that the strength of presynaptic inhibition should scale with the intensity of sensory input. Several strategies underlie the dynamic scaling of sensory feedback gain by GABApre neurons. At a circuit level GABApre neurons can be driven to high firing rates by proprioceptive input, and our physiological studies reveal a strict proportionality between the frequency of GABApre neuronal spiking and the strength of presynaptic inhibition. These two features suggest that GABApre neurons scale sensory input gain in register with proprioceptor firing frequency. At a synaptic level the prolonged time-course of presynaptic inhibition has the consequence that gain changes endure despite rapid fluctuation in sensory firing frequency over the course of a movement (Supplementary Note 3). The rapid spiking sustained by GABApre neurons also provides insight into the selectivity of expression of GAD2: by virtue of its association with synaptic vesicles GAD2 facilitates GABA release at high firing frequencies. Thus both the microcircuitry and neurochemistry of GABApre neurons contribute to the adaptive scaling of sensory feedback during motor control.

Motor circuits throughout the central nervous system are prone to oscillation. Yet the smoothness that normally characterizes limb trajectories implies that oscillations are suppressed before motor neuron activation. Behavioural studies, in conjunction with modelling, suggest that the GABApre gain control system provides one effective means of suppressing forelimb oscillation. We observe that mice at rest lack an overt tremor, consistent with the idea that oscillations are driven by proprioceptive feedback, a central tenet of the gain model. Moreover, the shallow relationship between gain and oscillation revealed by modelling offers a plausible explanation for the observed constancy of oscillation frequency and decay across GABApre-depleted mice. While the linear nature of the model explains primary oscillatory peak frequency, it leaves open the origins of sub-harmonic peaks. During periodic cycles of movement, sensory input occasionally fails to drive supra-threshold motor neuron activation, suggesting that sub-harmonics could have their basis in the intermittent skipping of one or more feedback-driven oscillatory cycles. Taken together, a simple model of sensory gain control provides theoretical support for the idea that GABApre neurons have a crucial role in ensuring smooth movement.

We note that the impact of manipulating presynaptic inhibition appears highly selective. Forelimb oscillations emerge after GABApre ablation despite the persistence of postsynaptic inhibition. Parallel theoretical and experimental analyses show that the subtractive nature of postsynaptic inhibition is far less effective in gain scaling than the divisive normalization achieved by presynaptic inhibition (Supplementary Discussion). Moreover, experimental inactivation of other defined spinal interneuron subtypes, both excitatory and inhibitory, impairs motor performance without obvious limb oscillation, implying a specialized contribution of presynaptic inhibition to smooth movement. Our findings also emphasize the modular nature of skilled reach, in that oscillations after GABApre ablation are evident only during the forelimb reach phase, leaving the initiation and later grasp phases unscathed. Such modular selectivity implies contextual recruitment of presynaptic inhibition during movement, permitting flexibility in the scaling of sensory gain (Supplementary Discussion). The precision of recruitment of GABApre neurons by sensory and descending pathways, when coupled with the diversity of sensory neurons influenced by presynaptic inhibition, hints at the existence of many GABApre neuronal subtypes, each devoted to gain control across discrete sensory feedback channels.
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Author Contributions A.F. and T.M.J. devised the project. A.F., E.A. and T.M.J. designed the experiments and analysed the data. A.F. performed the anatomical and electrophysiological experiments. E.A. developed the behavioural assay and with A.F. and K.R.C. performed the behavioural experiments. L.F.A. developed and with A.F. implemented the computational models. Z.H. generated the Gad2Cre mouse. A.F., E.A., L.F.A. and T.M.J. prepared the manuscript.

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METHODS

Mice. Gad2Cre-ER3 (Gad2cre) and H9:GFP (H9cre) mice were all on a C57BL/6 background. Animals were housed individually with light on 12:12 h cycle. For electrophysiology experiments, which required neonautes, Gad2Cre/cre homozygous mice were paired for breeding. Procedures performed in this study were conducted according to US National Institutes of Health guidelines for animal research and were approved by the Institutional Animal Care and Use Committee of Columbia University.

Viral vectors. To mark Cre-recombinase expressing neurons in Gad2cre mice for photoactivation or anatomical tracing, we used an adenovirus-associated viral (AAV) construct in which an inverted hCnr2 (H134B)-EYFP sequence was flanked by two pairs of heterotypic antiparallel loxP recombination sites (K. Deisseroth; Addgene plasmid 20298). AAV-FLEX-ChR2-YFP (FLEX-ChR2-YFP) was packaged and pseudotyped with AAV1 serotype to a titre of 1012 viral particles per ml (UNC Gene Therapy Center). For anatomical analysis and photoactivation experiments we performed FLEX-ChR2-YFP injection at p0–3 into lumbar spinal cord. For anatomical tracing of GABA-preneurons we performed FLEX-ChR2-YFP injection at p21–30 into lumbar spinal cord and p78 into cervical spinal cord.

To mark Gad2cre neurons for acute ablation we generated a Cre-dependent AAV-FLEX-DTR-GFP (FLEX-DTR-GFP), in which a simian DTR (HBEGF) sequence was fused to GFP. Details for the construction of the FLEX-DTR-GFP construct are described elsewhere. Briefly, the insert was flanked by two pairs of heterotypic, anti-parallel loxP recombination sites, downstream of a CAG promoter and upstream of a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) sequence. To confirm Cre-mediated expression specificity, the virus was packaged and pseudotyped with an AAV1 serotype to a titre of 5 × 1012 viral particles per ml (UNC Gene Therapy Center). To drive DTR expression in Gad2cre mice we performed FLEX-DTR-GFP injection at p56–84 into cervical spinal cord.

Lumbar viral injections for photoactivation and anatomical experiments. Gad2cre pregnant females were housed individually ~5 days before giving birth and given additional bedding materials to encourage nest building.

Before removing pups from the nest, the mother was placed in a separate cage so as not to be disturbed. All pups were removed from the nest and placed on a heating pad throughout the injection procedure. For injections, individual pups were anaesthetized with isoflurane. To gently stretch the vertebral column the feet and head of the pup were taped to a paper towel placed on a heating pad. A small, longitudinal incision was made in the skin between the hips and shoulder, directly under the right side of the C3–T1 segments, with three injection traces per segment, 25 nl per injection, spanning the dorso-ventral extent of the cord.

For ablation experiments, approximately 18–21 days following FLEX-DTR-GFP injection, mice were administered 400 ng of dipherthia toxin (Sigma-Aldrich) in sterile phosphate buffered saline via intraperitoneal injection. For analysis of ablation efficiency, mice were perfused one week after DT administration and tissue was processed for immunohistochemistry.

Electrophysiology, dissection. To obtain whole-cell patch-clamp recordings from motor neurons with intact sensory input and interneuron circuitry, we developed an in vitro semi-intact spinal cord preparation that permitted recordings from spinal motor neurons under visual guidance. Mice aged p9–14 were anaesthetized with tribromnoethanol (2.5% solution, 30 nl per gram body weight) and transcardially perfused using ice cold dissection artificial cerebral spinal fluid (dACSF) containing 234 mM sucrose, 3.6 mM NaCl, 1.2 mM Na2HPO4, H2O, 25 mM NaHCO3, 11 mM Na-glucose, 0.5 mM CaCl2, 4 mM MgCl2, 0.4 mM ascorbic acid, 2 mM pyruvate, 20 µM APV and 5 mM kynurenic acid, and equilibrated with 95% O2 and 5% CO2. We found that effective transcardial perfusion was critical to obtain healthy motor neurons at these ages.

Cervical viral injections for anatomical and ablation experiments. A detailed description of the cervical injection procedure can be found elsewhere. Briefly, for cervical spinal cord AAV injections, mice were anaesthetized with 0.01 ml g−1 body weight of 2.5% tribromnoethanol (Sigma-Aldrich) via intraperitoneal injection and maintained as needed. Eye lubricant was applied and the mice were placed in a stereotaxic frame (David Kopf Instruments). The head of the mouse was tilted forward and the tail was gently elevated and pulled back using a spinal vertebrae clamp (David Kopf Instruments). After making an incision in the skin covering the cervical spin cord, the cervical laminae were bared using fine bone forceps and a delicate bone scraper (Fine Science Tools). The laminae were cleaned with absorption sponges (Fine Science Tools) and gently separated via retraction of the large spinous process of the second thoracic vertebra using a small alligator clip held in place by a spinal vertebrae clamp. After removal of the cervical dura mater using fine forceps, FLEX-ChR2-YFP virus (for anatomical tracing) or FLEX-DTR-GFP virus (for ablation experiments) was delivered to cervical spinal cord using a Nanoject II. Injections were restricted to the right side of the C3–T1 segments, with three injection traces per segment, 25 nl per injection, spanning the dorso-ventral extent of the cord.

Before removing pups from the nest, the mother was placed in a separate cage so as not to be disturbed. All pups were removed from the nest and placed on a heating pad throughout the injection procedure. For injections, individual pups were anaesthetized with isoflurane. To gently stretch the vertebral column the feet and head of the pup were taped to a paper towel placed on a heating pad. A small, longitudinal incision was made in the skin between the hips and shoulder, directly under the right side of the C3–T1 segments, with three injection traces per segment, 25 nl per injection, spanning the dorso-ventral extent of the cord.

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Cervical spinal cord was then extracted and placed in a dissection dish superfused with oxygenated dACSF and cooled to ~2 °C using a custom made, chilled brass chamber (G. Johnson, Columbia University). The spinal cord and rib cage was pinned ventral side up in a Sylgard 184 Silicone Elastomer (Dow Corning)-coated dissection dish and the corpora were removed. The thoracic and sacral portions of the spinal cord were removed and a longitudinal incision was made in the dura mater along the ventral surface of the lumbar spinal cord using fine Vannas scissors (FST). Using no. 55 forceps (FST) the dura was then peeled off of the ventral surface on the right side of the spinal cord, taking care not to damage the dorsal roots. The cord was then hemisectioned by passing a bevelled crescent scapel (Sharpe) through the midline. The dorsal roots on the right side were then cut and the right half of the cord gently removed from the vertebral column. The left hemi-cord was used in some experiments for ventral and dorsal root recordings, coupled with photostimulation of the exposed midline, following standard methods.

To expose motor neurons for whole-cell recordings we removed the ventral laminae. To prepare the tissue for cutting, we embedded the cord in warm, LMP agar with a plastic transfer pipette. Once the cord was in the LMP agar, we slowly lowered the cord into 1.5% LMP agar, siphoning off the dACSF with a Pasteur pipette and applying the base mould (Fisher) and carefully positioned it so that the ventral surface was facing down, towards the base of the mould. We then quickly replaced the dACSF with 1.5% LMP agar, siphoning off the dACSF with a Pasteur pipette and applying the warm, LMP agar with a plastic transfer pipette. Once the cord was in the LMP agar, but before the agar had hardened, we carefully lifted the dorsal roots away from the cutting plane, making sure that the cord was positioned so that the dorsal–ventral axis was perpendicular to the bottom of the mould. We then placed the mould on a cold plate and covered the surface with a chilled glass coverslip to speed setting of the agar. It is important to place the mould on the cold plate only once the cord and roots are in position, as the agar sets quickly making further manipulation of the tissue difficult. The mould and coverslip were then immediately submerged in ice cold, oxygenated dACSF and the coverslip was removed once the agar was sufficiently hardened (after about ten seconds). Submerged in the solution, the agar block containing the hemiscord was then extracted from the mould using a small metal spatula.

The agar block was glued using Locite 404 cyanoacrylate glue to an angled block cut from 4% agar (~25° angle) which itself had been glued to the chilled vibratome stage. The stage was then quickly placed in the ice cooled vibratome bath. A vibratome (Leica VT1200) was used to remove the ventral-lateral white matter; the vibratome blade was positioned at the surface of the spinal cord, then lowered 50 µm below the surface and used to cut a sliver of white matter off of the hemiscord. Cuts were made, typically between one and three, until a fine strip of
grey matter was visible through a stereo dissection scope. The cut hemicord was then carefully removed from the 1.5% agar and placed in recovery solution. The hemicord was then allowed to recover for 30 min at 34 °C before being placed in the recording chamber (Warner) where it was superfused with recording ACSF containing 125 mM NaCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 2.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 0.4 mM ascorbic acid, 2 mM pyruvate and 26 mM D-glucose, and equilibrated with 95% O₂ and 5% CO₂. The recovery solution was a 50:50 mixture of ACSF and dASCF.

Electrophysiology recordings. The cord was placed in a laminar flow chamber (Warner) with the exposed dorsal surface facing up and secured with a harp, with fine, nylon fibres. Dorsal roots L3, L4 and L5 were positioned within polyethylene suction electrodes for nerve stimulation. The suction electrodes were held using a custom made holder and miniature manipulators (G.Z. Mentis, Columbia University). A stimulus isolator (AMPI) was used to stimulate dorsal roots with 100 μs current pulses. Dorsal roots were stimulated at 1.2-fold threshold, with threshold defined as the stimulation intensity that generated a postynaptic response in roughly 60% of trials. Stimulation intensities were typically between 2 and 10 μA, depending on the size of the root and the seal of the suction electrode. Whole-cell recordings were obtained using 2.5–3.5 MΩ pipettes pulled using a P-2000 puller (Sutter). An Olympus BX-51 microscope with an infrared DIC filter set was used to visualize motor neurons. A Multiclamp 700B amplifier and Digidata 1440A (Molecular Devices) were used to record and transfer electrical potentials to a desktop computer (Dell). Data were digitized at 20–50 kHz and a 10 kHz low-pass filter was used before digitization. Motor neurons were identified based on their ventral-lateral position, their large size and distinct dendritic morphology, their electrophysiological properties and their expression of GFP using HiyP26; transgenic mice. Motor neurons typically had a resting membrane potential between −68 and −74 mV. Cells with a resting membrane potential more depolarized than −60 mV were discarded.

Voltage clamp experiments were performed using a cesium-methanesulphonate-based internal solution containing 135 mM CsMeSO₃, 5 mM CsCl₂, 2 mM NaCl, 10 mM HEPES, 0.1 mM EGTA, 5 mM MgATP, 0.4 mM Na₃GTP, 10 mM phosphocreatine and 5 mM QX-314 chloride. Motor neurons were held between −60 and −70 mV and recordings in which the series resistance exceeded 20 MΩ or increased by greater than 20% during a recording were excluded. Because of the large size and low input resistance of the motor neurons, as well as the magnitude of the synaptic currents, series resistance was monitored but not compensated. Current clamp recordings were performed using a potassium-gluconate-based internal solution with 130 mM potassium gluconate, 5 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES and 4 mM NaATP. All experiments in which sensory-evoked synaptic currents were isolated were performed using 20 μM APV (Tocris). Internal solutions were titrated to a pH of 7.4 with potassium hydroxide for the potassium-gluconate-based internal solution and cesium hydroxide for the cesium-methanesulphonate-based internal solutions. The osmolality was 290 and 300 mOsm and was measured using a vapour pressure osmometer (Vapro).

To avoid potential confounds of voltage-gated channel activation during synaptic excitation, we applied internal blockage of voltage-gated potassium channels and the mean amplitude of light-evoked IPSCs in the presence of drug (either strychnine or SR 95531 and CGP 54626) and IPSC index is the mean amplitude of light-evoked IPSCs in the absence of drug:

\[
\text{IPSC index} = \frac{\text{IPSC}_{\text{drug}}}{\text{IPSC}_{\text{control}}}
\]

The value of the IPSC index is 1 when the drug has no effect and 0 when the drug completely blocks the IPSC. The value of the IPSC index is 1 when the drug has no effect on Gad₂⁻μ neuron-mediated suppression of sensory-evoked EPSCs and 0 when the drug abolishes the suppression of sensory-evoked EPSCs achieved by Gad₂⁻μ neuron photostimulation.

Vesicle depletion model. The vesicle depletion model was adapted from previously described simulations and was implemented in MATLAB. The model has three parameters: the time constant of facilitation, the time constant of depression and release probability. The facilitation time constant was not found to influence vesicle depletion at frequencies ±25 Hz, and so was set to 10 ms. The depression time constant was set so that normalized synaptic conductances generated by the model matched by eye those from control (no photostimulation) electrophysiological data. We found that depression time constants between 125 ms and 975 ms with a mean of 437 ± 305 ms s.d. captured the depression kinetics we observed. To determine the release probability time constant, the average amplitudes obtained from actual recordings. Changing the release probability parameter of the model, and only the release probability parameter, was sufficient to capture light-evoked changes in the rate of synaptic depression.

Concentrations. Data were collected in current clamp to avoid measurement errors introduced by changes in series resistance over long recording sessions (Extended Data Fig. 6). Each calcium concentration was visited twice for a given cell, and EPSP amplitudes used for analysis were taken from two sets of 25 consecutive dorsal root stimuli once the amplitude had stabilized following introduction of a new calcium concentration. Quadratic functions were fit using standard curve fitting routines in MATLAB. The \( C_{\text{v}} \) parameter (coefficient of variation) from EPSP amplitude from a single release site) in the mean variance analysis was set to 0.3, following established values. Changes (±10%) to this parameter did not markedly affect the release probability estimates.

Kinematic analysis of reaching. The basic training paradigm and reaching box design were largely based on established methods, and were adapted to 3D kinematic analysis in the mouse, as described.

Briefly, adult mice (12–20 weeks old) were food deprived to ~80–90% of their original body weight and habituated to target food pellets (20 mg, 3 mm diameter; Bio-Serv). Approximately 3 g of pellets were provided at the end of each...
day to maintain food deprivation. Following habituation, mice were placed into a clear acrylic box with a narrow opening. Food pellets were placed 1 cm away from the opening to the left of centre, to encourage use of the right arm. Mice were acclimated and trained to reach for three to four days, and the mice that were readily reaching with the right paw were selected for experiments (~50–75% of mice). Mice were then trained for two weeks for 20 min or 20 successful reaches each day, whichever occurred first.

For three dimensional quantification of reaching kinematics, mice were placed in a reaching box with a modified pellet tray in view of two high-speed, high-resolution monochrome cameras (HiSpec 1.2 GB memory; Fastec) with 50 mm f/1.4 manual iris and focus lenses (C-Mount, 1/2 inch CCD; Fujinon) placed ~80° apart. An infrared LED light source (Clover Electronics) was mounted on top of each camera, and each lens was equipped with an infrared long-pass filter (Midwest Optical Systems). Cameras were synced to each other, calibrated in space using MaxTRAQ 3D software (Innovision Systems) and set to 500 frames per second with a resolution of 1,280 × 1,024. A black background was used to increase contrast.

Mice were briefly anaesthetized with isoflurane, and a 1.5 mm reflective hemispheric marker (B & L Engineering) was attached to the back of the right paw with adhesive. HiSpec camera control software was used to record reach trials, using an external trigger in ring mode. MaxTRAQ 2D software (Innovision Systems) was used to automatically track the marker and the pellet, with trials beginning as the paw left the target and ending when the paw contacted the pellet to define a full reach and prehension motion was completed. Tracked files were imported into MaxTRAQ 3D software to compute the coordinates of the marker and pellet in three dimensions.

Data were imported into MATLAB, low-pass filtered, and the starting points of reach trials were normalized by discarding reaches that started at a distance (between paw and target) below 13 mm, and removing data frames for the remaining reaches above the 13 mm threshold (post-normalization: 112 DTR reaches, 75 control reaches; see ref. 31). Analyses of early reach and late grab phases were performed before and after a distance-to-pellet threshold of 11 mm, where the paw passes through the opening of the box. Mean power spectra were calculated using a fast Fourier transform of individual reach velocities. Decay times were calculated by aligning all reaches within individual mice to the maximum velocity peak of each reach, normalizing each reach to its maximum value, averaging across reaches and fitting a single exponential function to the first three estimated peaks for each mouse. The time constant of this exponential was considered the oscillatory decay time for each mouse. Power and decay analyses were performed on all reaches regardless of paw starting position, and thus non z-position normalized data were included (132 total DTR reaches). To estimate the initial and subsequent velocity peaks, the maximum velocity value was calculated for each reach in the first 50 ms, for the first peak, and the remainder of the reach, for the subsequent peak. Because all kinematic comparisons were performed within the same mouse before and after manipulation, and because tracking was automated, blinded analysis was not performed. Analyses were performed on approximately equal numbers of male and female mice selected randomly from trained populations, and no sex-specific differences in reaching success or kinematics were identified.

Reach success was quantified over a period of 20 min or 20 hits, whichever came first, for 10–20 total days beginning after recovery from viral injection, and for 5–6 days post-DT (beginning ~2 days after DT administration). Control mice were quantified for 14–24 days pre-DT and for 8–10 days post-DT (beginning ~3–7 days after DT administration)33.

Maximum digit abduction was calculated in MaxTRAQ 2D software by manually marking and measuring the distance between digits 2 and 4 in the frame captured by camera no. 1 in which the digits were maximally extended, as described31. The horizontal task was performed as described33. Briefly, a high-definition camcorder (Canon Vixia HF11) was used to capture locomotion across a walkway consisting of 13 evenly-spaced rungs. An angled mirror below the walkway provided a bottom-up view of the rungs. The number of successful (hits) and unsuccessful (mispsects) placements of the right and left forepaw were quantified for 10–15 full walks per mouse. The mean was calculated across all walks in individual mice pre- and post-DT. Pre-DT walks were recorded 1–2 weeks after viral injection and post-DT walks were recorded 5–7 days after DT administration.

For post-DT analysis of scratching, mice were placed in a clear box and the amount of time spent scratching the right forelimb and the rate of scratching bouts over a 10 min period were quantified. To prevent scratching-induced injury, following DT administration mice were anaesthetized with isoflurane and the claws of the right hindlimb were carefully removed with fine scissors. We observed no change in reaching behaviour due to hindlimb claw removal. To block cutaneous excitation and evaluate the effects on stretching, reaching and ladder paw placement, a topical lidocaine cream (DermPlanet; lidocaine, benzocaine, tetracaine) was applied to the right forearm and paw. Behavioural analysis resumed ~10–15 min after lidocaine application.

Joint model. We constructed a version of the Stein–Oğuztöreli model11 in which the angle of a single elbow-like joint (θ) is controlled by flexor (T_f) and extensor (T_e) muscle torques as follows,

$$\tau_m = \frac{d \theta}{dt} = T_e - T_f - Kr_m \frac{d \theta}{dt}$$

(3)

The relationship between joint angle and the dimensionless flexor and extensor torque defined in equation (3) is given by Newton’s second law in its angular form (where net torque is proportional to angular acceleration) combined with a damping term scaled by a dimensionless parameter k. Timescales in the model are set by a muscle time constant $t_m$ so that all variables and other parameters are dimensionless. We set the damping coefficient to $k = 0.5$, but found that our results were quite insensitive to the value of $k$ (see below and Extended Data Fig. 8).

To initiate joint extension, an excitatory drive ($I_e$) that peaks at 25 ms is applied to the dimensionless extensor torque in the form of a Gaussian pulse,

$$I_e = 25 \exp \left(\frac{(t - 250 \text{ ms})^2}{2(10 \text{ ms})^2}\right)$$

(4)

The width and magnitude of this pulse determine the magnitude of initial joint extension and were set by eye to match the time course of forepaw extension found in the reaching data. To capture sensory feedback signals generated by changes in joint angle, we included an angular velocity dependent feedback term coupled with a gain scaling factor $h$ that determines the efficacy with which sensory feedback drives flexor and extensor torques. This term, which is purely velocity dependent, introduces a drive of magnitude $s = h \tau m d \theta / dt$ into the muscle torque, where $s$ is the sensory feedback term. The relationship between extensor torque and sensory feedback is given by

$$\tau_m = \frac{d T_e}{dt} + 2 \tau_m \frac{d T_e}{dt} + T_e = I_e - h \tau_m \frac{d \theta}{dt}$$

(5)

which corresponds to an alpha-function muscle impulse response3. Here, the excitatory pulse $I_e$ serves to increase extensor torque and the sensory feedback term increases extensor torque when the joint’s angular velocity is less than zero, in other words when the joint angle is decreasing so the joint is flexing. Conversely, the relationship between flexor torque and sensory feedback is given by

$$\tau_m = \frac{d T_f}{dt} + 2 \tau_m \frac{d T_f}{dt} + T_f = I_e - h \tau_m \frac{d \theta}{dt}$$

(6)

Here the sensory feedback activates the flexor torque when the angular velocity of the joint is greater than zero, which occurs when the joint angle is increasing so the joint is extending. Note that there is no external drive to the flexor torque; its activation is achieved solely by sensory feedback.

In the original Stein–Oğuztöreli model, sensory feedback depends on both a muscle length term (analogous to joint angle) and a change of muscle length term (analogous to joint angular velocity), and there is a sensory feedback delay. We found that it was not necessary to include a joint angle dependence to observe joint oscillation at high feedback gains and so, for simplicity, we did not include these terms in our model. We also included feedback delays of varying latencies (Supplementary Note 5).

The model defined by equations (3), (5) and (6) has an instability when the gain $h$ exceeds a critical value $h_c$ that leads to ever-increasing oscillation amplitudes. We report all gains in relation to this critical gain $h_c$. To generate the traces included in Fig. 6c, d, we set sensory feedback gain at two different levels. For the low-gain case, we set $h$ to 10% of the critical gain ($h_c = 0.1$). Under this condition, the external extensor drive triggers joint extension, which is arrested by flexion activated by sensory feedback. The magnitude of the sensory feedback generates flexor torque of sufficient strength to arrest joint extension but of insufficient strength to generate further extensor torque. The velocity oscillation (Fig. 6c) is, in other words, near to being critically damped. In contrast, in the high-gain case, where $h$ is 60% of the critical gain ($h_c = 0.6$), the sensory activated flexor torque is of sufficient strength to activate an additional extensor torque, via a pronounced and rapid decrease in joint angle. This extensor torque, in turn, triggers an increase in joint angle of sufficient velocity to trigger another flexor torque and oscillation ensues (Fig. 6d).

In the high-gain case, to achieve continuous joint extension in the phase of oscillation we add a constant value to the extensor drive, setting $I_e \rightarrow I_e + 10$. We incorporated varying delays into the model by defining the contribution of the sensory feedback with a delayed angular velocity term such that for $t > t_{delay}$, $\theta(t) = h \tau_m (t - t_{delay})$, where, in individual simulations, $t_{delay}$ is taken from a set of delay values (1, 3, 5 and 10 ms) as reported in Extended Data Fig. 8. For multiple delays, we summed $\frac{d \theta}{dt}$ evaluated at different times, with equal weighting.

To simulate joint extension in the absence of subtractive scaling (representing postsynaptic inhibition) we added a constant value to the sensory feedback such

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that $s = h\tau_m \frac{dt}{dt} + C$. The added constant $C$ represents the added feedback that would emerge in the absence of a tonic subtractive postsynaptic inhibitory term. We set $C = 0$ for $\frac{dt}{dt} \leq 0$. Without this rectification, the sensory feedback generates a constant flexing torque that destabilizes the resting state of the arm. We note that even in this case there is no oscillation in the joint, but to compare its behaviour with reduced subtractive inhibition to cases without this added constant, we included the rectification step. We note that this is the only rectification in our model. In general, to keep the model linear, we did not sign-constrain either the stretch response or the muscle torques. We checked the behaviour of the model with sign-constrained stretch responses and muscle torques and note that the basic oscillatory behaviour persists at high gain, with near to critical damping at low gain.

To analyse the oscillatory behaviour of the model, we expressed the equations that describe the dynamics of the model (equations (3), (5) and (6)) as a system of five, first-order, linear differential equations:

$$
\begin{pmatrix}
-\kappa & 1 & -1 & 0 & 0 \\
0 & -1 & 0 & 1 & 0 \\
0 & 0 & -1 & 0 & 1 \\
-h & 0 & 0 & -1 & 0 \\
h & 0 & 0 & 0 & -1
\end{pmatrix}
\begin{pmatrix}
\tau_m \frac{dt}{dt} \\
\tau_e \frac{dt}{dt} \\
T_i \frac{dt}{dt} \\
z_e \\
z_l
\end{pmatrix}
= 
\begin{pmatrix}
z_e \\
z_l
\end{pmatrix}
$$

(7)

where $z_e$ and $z_l$ are variables that permit the two second-order equations (5) and (6) to be expressed as four first-order equations. Computing the eigenvalues of the matrix describing this system of linear equations permits a description of the frequency and damping time of its dynamic modes. Three of the eigenvalues are real, corresponding to decaying modes. The eigenvalue with the least negative real part, which we call $\lambda_1$, is complex (actually it is one of a complex-conjugate pair of eigenvalues), corresponding to the dominant oscillatory mode of the model. The damping time of the oscillation is determined by the real part of $\lambda_1$ and the frequency $f_{osc}$ of the oscillations by the imaginary part of $\lambda_1$. The damping describes the rate at which the oscillations decay. There is a level of gain above which the damping time approaches infinity and oscillations do not decay, which defines the critical gain $h_c$.

To determine the appropriate values of the additional model parameters $h$ and $\tau_m$, we fit the oscillatory frequency and decay time of the leading eigenmode of the model to the values measured from post-DT reaching movements across mice (Extended Data Fig. 8a, b and Supplementary Note 4). The frequency of oscillation of the model is

$$f_{osc} = \frac{|\text{Im}(\lambda_1)|}{2\pi\tau_m}$$

(8)

where the numerator is the imaginary part of the complex eigenvalue corresponding to the model’s oscillatory mode. The decay time of oscillation is,

$$\tau_{osc} = \frac{\tau_m}{|\text{Re}(\lambda_1)|}$$

(9)

where the denominator is the real part of the complex eigenvalue corresponding to the model’s oscillatory mode. The product of these two values generates a term $n_{cycle}$ that is independent of the muscle time constant (the muscle time constant cancels when the right side of equation (8) is multiplied by the right side of equation (9)),

$$n_{cycle} = \frac{|\text{Im}(\lambda_1)|}{2\pi|\text{Re}(\lambda_1)|}$$

(10)

Here $n_{cycle}$ corresponds to the number of oscillatory cycles within a decay time constant. Since we have measured the frequency and decay time of post-DT oscillation, we calculated an experimentally-derived value of $n_{cycle}$. We then used this value to determine which sensory gain level corresponds to the reach oscillations we observed. We also examined the extent to which the drag term $k$ affects $n_{cycle}$, varying $k$ across a 100-fold range and finding minimal change in the gain value that corresponds to our experimentally-derived $n_{cycle}$ value (Supplementary Note 4 and Extended Data Fig. 8a). We then used this gain value to estimate the muscle time constant (Supplementary Note 4 and Extended Data Fig. 8b). We found a biologically realistic value that was within the range used in the original model.

**Antibodies.** Primary antibodies: rabbit anti-GFP (1:500, Invitrogen); sheep anti-GFAP (1:1,000, ABD Serotec); rabbit anti-idT (1:1,000, Clontech; Living Colors DdRed); custom made guinea pig anti-vGluT1 (1:32,000); rabbit anti-GAD1 (1:10,000); and rabbit anti-GAD2 (1:10,000) were used as described previously. Appropriate fluorophore-conjugated secondary antibodies were from the Jackson ImmunoResearch antibody series.

**Immunohistochemistry and imaging.** Mice were transcendarily perfused with 4% paraformaldehyde 0.1 M phosphate buffer fixative. For cryostat spinal cord sections, tissue was post-fixed for 2 h, washed, equilibrated in 30% sucrose 0.1 M phosphate buffer solution, embedded and frozen in O.C.T. and sectioned along the axial plane at 20 μm onto glass slides. Immunohistochemistry was performed via exposure to primary antibodies (overnight at 4 °C) and fluorophore-conjugated secondary antibodies (1 h at room temperature). Sections were mounted using Fluoromount-G (SouthernBiotech) and coverslipped for imaging. Confocal images were taken with an LSM 710 microscope (Carl Zeiss).

**Statistics.** Results are expressed as the mean ± s.e.m. or s.d., as indicated. Electrophysiology data were analysed using two-tailed paired $t$-tests. For behavioural experiments early tests revealed very large effects of GABApre ablation (for example see Extended Data Table 1); power analysis demonstrated that with these effect sizes relatively small sample numbers are sufficient to achieve a power of value 0.8 with an alpha value of 0.05. The large effects observed mitigate potential confounds introduced by possible violations of parametric test assumptions. Reach success and kinematic data were analysed using two-way repeated-measures ANOVA, enabling within-mouse pairing of data. Successful and unsuccessful reaches were grouped together for pre-DT conditions. There were no successful reaches following GABApre neuronal ablation in the kinematic assay. Bonferroni post hoc multiple comparisons tests were performed, as indicated. DT-treated control mice spared viral injection were analysed in parallel experiments and were used in two-way repeated-measures ANOVA analysis. For velocity power spectra analysis, lidocaine scratching analysis, digit ablation and ladder walk data, two-tailed paired $t$ tests were used. $P < 0.05$ was considered significant.

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Extended Data Figure 1 | Genetic targeting of GABApre neurons in lumbar spinal cord. a, At late ages (p30) FLEX-ChR2-YFP lumbar injection in Gad2Cre mice marks GABApre neurons. In ventral horn (yellow box): YFP+/GAD2+ GABApre boutons contact vGluT1+ proprioceptor terminals (b, higher magnification). c, YFP+/GAD1+ GABApost boutons do not contact vGluT1+ terminals. In contrast, a single YFP+/GAD1+ bouton is in contact with a vGluT1+ terminal and is, therefore, a GABApre bouton. p30 injection marks GABApre boutons (75.8 ± 3.3%) but not GABApost boutons (0.99 ± 0.02%; n = 2). Values indicate mean ± s.e.m.
Extended Data Figure 2 | Light-evoked excitation of spinal motor neurons reflects Gad2loxP neuron-mediated proprioceptor depolarization.

a, Recordings from dorsal and ventral roots during Gad2loxP neuron photostimulation at 24–26 °C. b, Light pulses induced primary afferent depolarization (PAD, top), accompanied by antidromic action potentials (black arrow), in dorsal roots shortly followed by orthodromic discharge in ventral roots (bottom). Outline indicates region shown in c. As shown in Fig. 2f, the time course and amplitude of PAD evoked by sensory stimulation and Gad2loxP-neuron photostimulation are similar. Sensory-PAD: amplitude 203 ± 2 μV; 10–90% rise time 18.8 ± 0.3 ms; 10–90% decay time 275.7 ± 8.3 ms; n = 10 trials. Gad2loxP-evoked PAD: amplitude 230 ± 1 μV; 10–90% rise time 17.3 ± 0.3 ms; 10–90% decay time 186.6 ± 2.0 ms. c, Mean antidromic spike recorded in dorsal root (top) and orthodromic spike recorded in ventral root (bottom). The latencies of spike onset (outlined region) are shown on the right (dorsal root spike onsets, blue circles; ventral root spike onsets, brown circles; vertical lines indicate latencies from light pulse onset). Ventral root spike onset was consistently later than dorsal root spike onset (mean delay 0.73 ± 0.03 ms; mean dorsal root spike latency 8.01 ± 0.42 ms; mean ventral root spike latency 8.74 ± 0.40 ms; two-tailed paired t test, P < 0.05, n = 2 preparations), indicating that light-evoked spikes occur in sensory neurons before motor neurons. d, Whole-cell patch-clamp recording from spinal motor neurons during Gad2loxP neuron photostimulation.

e, Light-induced excitatory postsynaptic potentials (EPSPs) (1 ms pulses) evoked at 24 (left) but not 30 °C (right). Inset, left, temperature dependence of light-evoked EPSPs (top plot, bath temperature (T); bottom plot, EPSP amplitudes; both plotted as functions of time; note EPSP recovery with return to low temperature). Inset, right, average EPSP amplitude as a function of bath temperature. f, During low temperature (24–26 °C) recording conditions, application of the GABA-A receptor antagonist gabazine (SR 95531, Gbz, 2 μM) abolishes light-evoked EPSPs but leaves the predominantly glycinergic light-evoked inhibitory postsynaptic potential (IPSP) intact. g, During low temperature (24–26 °C) recording conditions, application of the AMPA-receptor antagonist NBQX (2 μM) abolishes the fast, high-amplitude component of the light-evoked EPSP but does not affect the light-evoked IPSP. Inset, individual EPSP amplitudes during gabazine application (Gbz, grey bar).

Together these experiments are consistent with the view that synchronous activation of Gad2loxP neurons at low temperature depolarizes sensory afferent terminals with sufficient strength to generate sensory action potentials and subsequent glutamate release, in turn activating motor neurons (Supplementary Note 2). Values and error bars indicate mean ± s.e.m.
Extended Data Figure 3 | Physiological identification of spinal motor neurons. a, Whole-cell patch-clamp recording from spinal motor neurons in a whole (or hemisected) cord, in which motor neurons were targeted via visual guidance using GFP expression in Hb9GFP mice. b, Removal of the ventral-lateral white matter (see Methods) permits visual identification and access to spinal motor neurons without disrupting sensory–motor or interneuron circuitry. c–e, Motor neurons recorded in this configuration exhibit physiological properties typically associated with spinal motor neurons56: c, current injection elicits repetitive action potentials, with rate adaptation; d, hyperpolarizing current steps reveal membrane potential sag, indicative of $I_h$ current; and e, the waveform of motor neuron action potentials exhibits an early hyperpolarization indicative of an $I_A$ current and a prolonged hyperpolarization likely mediated by a calcium-activated $I_{Ca(Ca)}$ current.
Extended Data Figure 4 | Gad2Cre neuron photoactivation scales sensory EPSC amplitude.  

a, Sensory-evoked EPSCs observed in motor neurons at varying delays (Δt) between the cessation of photostimulation (ten 1 ms pulses, 100 Hz, blue line) and sensory stimulation (black line). Fractional EPSC amplitude (EPSC/EPSC) plotted as a function of sensory delay Δt. Maximal EPSC suppression was seen at the smallest Δt values. Plot depicts fractional EPSC amplitude for Δt = 35, 60, 110, 135, 260, 510, 760 and 1,010 ms.

b, Sensory-evoked EPSCs following a varying number of light pulses (Σλ, blue line) at a fixed latency (Δt = 45 ms) recruits Gad2Cre neuron-evoked EPSC suppression of increasing magnitude. Plot depicts fractional EPSC amplitude for Σλ = 1–14 pulses.

c, Sensory-evoked EPSCs following varying Gad2Cre neuron photoactivation frequency (fL, blue line) at a fixed sensory delay (Δt = 45 ms) recruits increasing suppression of sensory-EPSCs. Plot depicts fractional EPSC amplitude for fL = 10, 15, 20, 25, 50 and 100 Hz. These data, along with the linear scaling of Gad2Cre neuron firing with photoactivation frequency (Fig. 2d), indicate that the graded suppression of sensory-EPSCs is a consequence of increased spiking in Gad2Cre neurons. Red curves indicate splines fit to the data. Error bars indicate mean ± s.d. We note that these experiments require effective control of Gad2Cre neuronal spiking. As shown in Fig. 2b–d, light pulses induced pronounced currents and reliable spiking in ChR2–YFP Gad2Cre neurons (Vholding = −60 mV; peak 773 ± 268 pA; steady state 537 ± 193 pA; 10–90% rise time 2.1 ± 0.4 ms; 10–90% decay time 33.5 ± 4.3 ms; n = 3). Values indicate mean ± s.e.m.
Extended Data Figure 5 | Postsynaptic inhibition cannot account for the observed reduction in sensory-evoked EPSC amplitude. a, Motor neuron IPSC (mean of ten trials) elicited by a 1 ms light pulse. IPSCs had a 10–90% decay time of 23.7 ± 1.9 ms and a mean peak amplitude of 299 ± 20 pA at 
260 mV holding potential (n = 9). IPSCs reversed at 265 mV (Nernst equation predicts ECl = 267.5 mV). b, A 45 ms delay between the light pulse (blue) and electrical sensory stimulation pulse (black) permits the GABApost-mediated IPSC to decay almost completely before sensory-EPSC onset. c, Gad2Cre neuron-evoked IPSCs are monosynaptic. Red circles indicate estimated onset times with distribution of individual IPSC latencies shown in inset. Mean IPSC onset latency 2.4 ± 0.3 ms; cv onset 0.03 ± 0.007; n = 5. As shown in Fig. 3b–d, IPSCs are almost completely blocked by strychnine (Str), with only limited effects of GABA receptor antagonists (Gbz/Cgp). IPSC index, ratio of IPSC amplitude with drug to control IPSC (Str 0.09 ± 0.02, two-tailed paired t test, P < 0.001, n = 5; Gbz/Cgp 0.82 ± 0.04, P < 0.05, n = 3). Moreover, as shown in Fig. 3e–g, the Gad2Cre neuron-evoked suppression of sensory-evoked EPSCs is unaffected by strychnine (Str) but abolished by co-application of the GABA-A and -B receptor antagonists SR 95531 and CGP 54626. EPSC index, drug has no effect (51; drug abolishes EPSC-suppression = 0 (Str 1.04 ± 0.02, two-tailed paired t test, P = 0.3, n = 3; Gbz/Cgp 0.02 ± 0.03, two-tailed paired t test, P < 0.02, n = 4; see Methods). d, Sensory-evoked EPSC (black trace, control) and EPSCc (blue trace, with Gad2Cre photoactivation) waveforms fit with double exponential functions (EPSCc = A1 exp(−rt1) + A2 exp(−rt2)). e, Distribution of the fast decay time constant (t1) is similar for EPSC (black circles) and EPSCc (blue circles) waveforms (EPSC t1 96 ± 13 µs; EPSCc t1 89 ± 14 µs; n = 100 trials). f, Distribution of the slow decay time constant (t2) is similar for EPSC and EPSCc waveforms (EPSC t2 5.56 ± 0.41 ms; EPSCc t2 5.41 ± 0.48 ms). g, Estimation of the 20–80% rise and decay time provides an independent measure of EPSC kinetics. h, The 20–80% rise time for control EPSCs (EPSC 199 ± 7 µs) and that of EPSCs evoked following photoactivation (EPSCc 197 ± 9 µs). i, The 20–80% decay time for control EPSCs (EPSC 7.0 ± 0.5 ms) and that of EPSCs evoked following photoactivation (EPSCc 7.3 ± 0.6 ms). j, Normalization of the light-conditioned EPSCc waveform to the unconditioned EPSC waveform permits estimation of waveform correlation as a further metric of the similarity of the two waveforms (Pearson’s correlation, R = 0.999, P < 0.0001). The unaltered time course of the EPSC waveform argues against light-evoked EPSC suppression mediated by a postsynaptic inhibitory conductance. Although in theory a postsynaptic inhibitory conductance should have no effect on the current waveform of a perfectly clamped EPSC, the large size and low input resistance of motor neurons, as well as the dendritic nature of the majority of sensory inputs, makes it likely that the voltage clamp is insufficient to prevent some depolarization of the dendrites, which would be altered via inhibitory conductance. In support of this idea the time course of EPSP waveforms recorded in current clamp are unaltered by photoactivation of Gad2Cre neurons under the conditions described here (data not shown). Values and error bars indicate mean ± s.e.m. in a–c and mean ± s.d for d–j.
Extended Data Figure 6 | Estimation of release probability using mean variance analysis validates the short-term depression model. a, Amplitudes of 225 EPSPs recorded from a spinal motor neuron evoked by sensory stimulation across five calcium concentrations (0.5, 1.0, 1.5, 2.0 and 4.0 mM) exhibiting fluctuating amplitudes due to calcium-controlled changes in transmitter release probability. EPSPs were recorded at each calcium concentration (0.1 Hz sensory stimulation frequency) once EPSP amplitude had settled to a steady value. Each calcium concentration was visited twice in sequence per recording. Data were collected in current clamp to reduce possible confounds due to changes in series resistance over the long recordings required to collect the data. b, EPSP variance versus amplitude (black circles) is well fit by a quadratic function (grey line) of the form \( s^2 = A\mu + B\mu^2 \), where \( s^2 \) is the variance across EPSPs and \( \mu \) is the mean EPSP amplitude for each calcium condition. The release probability \( (p_r) \) is given by \( p_r = \mu (B/A) (1 + cv_q^2) \), where \( cv_q \) is the coefficient of variation in EPSP amplitude from a single release site. We set \( cv_q = 0.3 \), following accepted values. Using this method, we found that for the representative neuron shown here \( p_{r1} = 0.97, 0.86, 0.66, 0.35 \) and 0.07 for \([Ca^{2+}]_o = 4, 2, 1.5, 1 \) and 0.5 mM, respectively. c, At each calcium concentration EPSP depression was monitored via repetitive stimulation of the dorsal root (10 pulses, 10 Hz). Shown here are five consecutive EPSPs for three calcium concentrations (1.5, 1.0 and 0.5 mM; superposition of six trials). d, Changes in EPSP depression as a function of calcium concentration were quantified using a model of short-term depression (see Fig. 3h–j). The short-term depression model was set to generate EPSP amplitudes that depressed at different rates because of changes to the release probability term of the model. These EPSP amplitudes were fit by exponential functions. Normalized mean EPSP amplitudes at different calcium concentrations (circles) were then superimposed upon the curves generated by the short-term depression model (grey lines). The depression shown here is captured by setting the release probability parameters in the short-term depression model to \( p_{r1} = 0.52, p_{r2} = 0.30 \) and \( p_{r3} = 0.07 \) for \([Ca^{2+}]_o = 1.5, 1.0 \) and 0.5 mM, respectively, which are in good correspondence with the release probability terms estimated using mean variance analysis. For \([Ca^{2+}]_o > 1.5 \) mM the short-term depression model consistently produced lower estimates for release probability than the mean variance analysis. But the correspondence at 1.5 mM and below argues for the short-term depression model being an effective means of capturing changes in release probability at sensory-motor synapses.
Extended Data Figure 7 | Reach trajectories before and after ablation of GABA\textsubscript{pre} neurons. a, Pre-DT reach trajectories for four mice. Two dimensional paw trajectories were generated by projecting the three dimensional trajectory into the $x$–$z$ plane (see Fig. 5a). Green traces represent pre-DT hits, blue traces, pre-DT misses. The asterisk indicates the location of the target food pellet. b, Post-DT two dimensional reach trajectories for the same mice, red traces. These mice were used for all kinematic quantification included in Extended Data Table 1.
Extended Data Figure 8 | Fitting the model to the frequency and decay time of post-DT reach velocity oscillations. a, To fit the model parameters to the experimentally-derived frequency and decay times, we took advantage of the fact that the product of the oscillatory frequency and decay time ($f_{osc} \cdot \tau_{osc}$) creates a parameter free value $n_{cycle}$, which does not depend on the muscle time constant. The term $n_{cycle}$ corresponds to the number of oscillatory cycles per decay time constant and scales as a function of feedback gain. Plotting $n_{cycle}$ as a function of feedback gain ($h/h_c$, where $h_c$ is the critical gain above which oscillations do not decay) permits estimation of the gain value that corresponds to the experimentally-derived value of $n_{cycle}$. Importantly, the corresponding gain value does not depend on the drag parameter $k$ (inset and grey shading beneath black curve). Varying $k$ by 100-fold results in minimal changes in the corresponding gain, with $h/h_c$ varying from 0.60 to 0.62. The grey curves represent the relationship between $n_{cycle}$ and $h/h_c$ across the full range of $k$ values. Arrow indicates the gain value corresponding to the experimentally-derived value of $n_{cycle}$ for $k = 0.5$. See Supplementary Note 4 and Methods. b, To estimate the appropriate muscle time constant ($\tau_m$), we used the five dimensional matrix that defines the model (see Methods) to calculate the imaginary part of the complex eigenvalue corresponding to $k = 0.5$ and $h/h_c = 0.61$. We found a time constant of $\tau_m = 9.6$ ms (indicated by the arrow), which is within the range used in the original model based on experimentally-derived values. c, Introduction of delays in the feedback loop does not alter the basic properties of the model. Oscillation lifetime ($\tau_{osc}$) as a function of absolute gain $h$ for delays of 1, 3, 5 and 10 ms as compared to no delay (black trace). The vertical dashed lines indicate the gain level above which oscillations never decay (the critical gain, $h_c$) for each delay value. d, Scaling these curves by their corresponding critical gain values ($h/h_c$) reveals equivalent oscillatory lifetimes. e, Oscillation frequency ($f_{osc}$) as a function of normalized gain. As feedback delays increase, peak oscillatory frequency decreases. For higher delay conditions, where the oscillation frequency is significantly below 20 Hz, decreasing the muscle time constant results in higher oscillation frequencies. Thus for longer delays, 20 Hz oscillation is possible, but requires an increasingly small muscle time constant. f–h are as c–e but with three simultaneous loops of different delays (1, 3 and 5 ms). In the presence of multiple simultaneous feedback delays the model continues to oscillate at a single dominant peak frequency for a given gain level. See Supplementary Note 5 and Methods.
Extended Data Figure 9 | Lidocaine application abolishes scratching behaviour but not reach oscillations, and does not affect forepaw stepping behaviour. **a**, Scratching behaviour increased following GABApre neuronal ablation, but was reduced to normal levels by topical application of lidocaine to the right forearm and paw. The rate of scratching bouts per 10 min observation session increased following DT-administration, but returned to normal levels following lidocaine application (mean rate of scratch bouts per minute: pre-DT 0.6 ± 0.2 min⁻¹; post-DT 5.1 ± 0.9 min⁻¹; post-DT plus lidocaine 1.0 ± 0.5 min⁻¹; two-tailed paired t-test, pre-DT versus post-DT, P < 0.05; post-DT versus post-DT + lidocaine, P < 0.05, n = 4). **b**, Similarly, the percent time spent scratching during a 10 min observation period is normally low (pre-DT 1.6 ± 0.4%) but increased following DT-administration (post-DT 49.8 ± 11.7%) and trended towards baseline after lidocaine application (post-DT plus lidocaine 2.9 ± 1.3%). Two-tailed paired t-test, pre-DT versus post-DT, P < 0.05; post-DT versus post-DT + lidocaine, P = 0.06, n = 4. **c**, Stepping success rate of the right forepaw on the horizontal ladder task. Lidocaine did not affect stepping performance (pre-DT 90 ± 1%; post-DT 93 ± 1%; post-DT + lidocaine 92 ± 1%; two-tailed paired t-test, pre-DT versus post-DT, P = 0.7; post-DT versus post-DT + lidocaine, P = 0.6, n = 3). The equivalent rate of accuracy across conditions indicates that lidocaine application has no overt effect on forepaw placement during stepping. As shown in Fig. 4f, right and left forepaw placement accuracy pre-DT versus post-DT were similar (right paw: pre-DT 90.2 ± 2.0%; post-DT 79.6 ± 13.2%; two-tailed paired t-test, P = 0.41; left paw: pre-DT 80.8 ± 2.7%; post-DT 76.1 ± 6.9%; two-tailed paired t-test, P = 0.34, n = 4). In contrast to stepping accuracy, as shown in Fig. 4e, reach success degraded following GABApre ablation as compared to control mice (pre-DT 48.6 ± 3.7%; post-DT 4.9 ± 4.7%; two-way repeated-measures ANOVA, interaction of group × toxin: F₁,₆ = 17.64, P = 0.006; post hoc Bonferroni test, DTR: P < 0.01, n = 4 DTR, n = 4 control). **d**, Post-DT velocities of individual reaches from a representative mouse continued to exhibit oscillation following topical lidocaine application. **e**, Power spectrum of post-DT reaches following lidocaine application (n = 2 mice, 14 reaches; shaded area, s.d.). Mean dominant frequency peak for post-DT reaches with lidocaine (20.5 ± 3.8 Hz) and without lidocaine (19.5 ± 0.5 Hz; see Fig. 5f,g). The persistence of limb oscillation following lidocaine block implicates a loss of proprioceptive rather than cutaneous presynaptic inhibition as the origin of the oscillation uncovered by GABApre neuronal ablation. Values and error bars indicate mean ± s.e.m.
**Extended Data Table 1 | Reaching kinematics after GABApre neuronal ablation**

|                        | Reach phase | Grab phase |
|------------------------|-------------|------------|
|                        | Pre-DT | Post-DT | Pre-DT | Post-DT |
| **Direction reversals (no.)** | 1.38 ± 0.30 | 13.36 ± 1.36 | 1.72 ± 0.12 | 1.18 ± 0.08 |
| **Direction reversals at >100 mm s⁻¹ (no.)** | 0.07 ± 0.02 | 5.68 ± 0.46 | 0.02 ± 0.01 | 0.10 ± 0.03 |
| **s.d. of distance to target (mm)** | 2.93 ± 0.24 | 4.89 ± 0.25 | 0.91 ± 0.09 | 0.92 ± 0.05 |
| **s.d. of velocity (mm s⁻¹)** | 36.34 ± 1.80 | 92.05 ± 6.80 | 35.20 ± 2.10 | 48.11 ± 2.24 |
| **Total distance travelled per reach (mm)** | 26.87 ± 0.44 | 84.67 ± 3.14 | 12.63 ± 0.40 | 8.03 ± 0.31 |
| **Total duration per reach (ms)** | 255.20 ± 7.84 | 582.93 ± 47.24 | 116.47 ± 6.80 | 65.78 ± 3.96 |
| **Mean reach speed (mm s⁻¹)** | 113.79 ± 5.80 | 157.99 ± 12.03 | 106.83 ± 4.27 | 133.25 ± 5.64 |

**Entire reach**

|                        | Pre-DT | Post-DT |
|------------------------|--------|--------|
| **Initial peak velocity at <50 ms (mm s⁻¹)** | 296.96 ± 16.73 | 284.14 ± 50.61 |
| **Subsequent peak velocity at >50 ms (mm s⁻¹)** | 161.80 ± 10.36 | 274.17 ± 29.06 |
| **Peak power (18–22 Hz)** | 143 ± 37 | 957 ± 270 |

We observed an increase in the following kinematic parameters in the reach, but not the grab phase in GABApre-ablated relative to control mice: mean number of forepaw direction reversals towards and away from the pellet (n = 4 DTR mice, 112 reaches, n = 4 control mice, 75 reaches; two-way repeated-measures ANOVA, interaction of group × condition, reach phase: F₁,₆ = 15.48, P = 0.01; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001; grab phase: F₁,₆ = 3.24, P = 0.12); mean number of high velocity (>100 mm s⁻¹) reversals of paw direction (reach phase: F₁,₆ = 35.04, P = 0.001; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001; grab phase: F₁,₆ = 2.71, P = 0.15); mean s.d. of paw distance to target (reach phase: F₁,₆ = 8.57, P = 0.03; grab phase: F₁,₆ = 0.60, P = 0.47); mean s.d. of paw velocity (reach phase: F₁,₆ = 25.55, P = 0.002; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001; grab phase: F₁,₆ = 1.12, P = 0.33); the mean total distance travelled by the paw (reach phase increase in distance travelled: F₁,₆ = 62.42, P = 0.0002; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.0001; grab phase decrease in distance travelled: F₁,₆ = 15.57, P = 0.01; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001); and mean movement duration (reach phase increase in duration: F₁,₆ = 12.67, P = 0.01; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001; grab phase decrease in duration: F₁,₆ = 13.14, P = 0.01; post hoc Bonferroni test, DTR pre-DT versus post-DT, P < 0.001). Mean reach speed did not differ significantly between conditions. Digit abduction (maximum distance between digits 2 and 4) during grasp attempts was unaffected by GABApre neuronal ablation (P = 0.98, n = 5). Reaching kinematics were not affected during any phase of movement in DT-treated control mice that did not receive FLEX-DTR-GFP. As shown in Fig. 5d, the amplitude of the first velocity peak (within the first 50 ms of the reach) did not differ between pre-DT and post-DT reaches, while the subsequent peak velocity (>50 ms from reach initiation) was significantly higher for post-DT reaches (initial peak: pre-DT versus post-DT, two-tailed paired t test, P = 0.82; subsequent peak: pre-DT versus post-DT, two-tailed paired t test, P = 0.64, n = 4 mice, 112 reaches). As shown in Fig. 5g, peak power in the 18–22 Hz frequency band was significantly higher in post-DT mice (two-tailed paired t test, P = 0.02, n = 5 mice, 132 reaches). Successful and unsuccessful pre-DT reaches were grouped together for all analyses. There were no post-DT successful reaches in the kinematic assay. Values indicate mean ± s.e.m.