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Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT_{2C} receptors

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Muscle paralysis after spinal cord injury is partly caused by a loss of brainstem-derived serotonin (5-HT), which normally maintains motoneuron excitability by regulating crucial persistent calcium currents. Here we examine how over time motoneurons compensate for lost 5-HT to regain excitability. We find that, months after a spinal transection in rats, changes in post-transcriptional editing of 5-HT_{2C} receptor mRNA lead to increased expression of 5-HT_{2C} receptor isoforms that are spontaneously active (constitutively active) without 5-HT. Such constitutive receptor activity restores large persistent calcium currents in motoneurons in the absence of 5-HT. We show that this helps motoneurons recover their ability to produce sustained muscle contractions and ultimately enables recovery of motor functions such as locomotion. However, without regulation from the brain, these sustained contractions can also cause debilitating muscle spasms. Accordingly, blocking constitutively active 5-HT_{2C} receptors with SB206553 or cyproheptadine, in both rats and humans, largely eliminates these calcium currents and muscle spasms, providing a new rationale for antispastic drug therapy.

Severe spinal cord injury (SCI) causes an immediate paralysis of muscles innervated by motoneurons directly caudal to the injury site. This results not only from a loss of supraspinal tracts that subserve voluntary initiation of movement (for example, corticospinal and reticulospinal tracts that use fast glutamatergic synaptic transmission[1,2]) but also from a loss of descending brainstem tracts that provide spinal motoneurons with their major source of neuromodulators, such as 5-HT (refs. 1,3–5). Normally, brainstem-derived 5-HT sets spinal motoneurons and interneurons into an excitable state, ready to respond to fast glutamate synaptic inputs and cause appropriate muscle contractions[2,4,6,7]. 5-HT does this by activating 5-HT_2 receptors that facilitate ionic currents intrinsic to the motoneurons, including voltage-gated persistent Ca^{2+} and Na^+ currents (termed persistent inward currents: PICs)[1,8–11]. These PICs are easily activated by brief synaptic inputs because of their unusually low threshold and, thus, serve a crucial role in amplifying and prolonging the action of synaptic inputs, ultimately enabling sustained muscle contractions[2,7,11–14]. Consequently, when SCI eliminates brainstem-derived 5-HT, motoneurons are left in an unexcitable state with small PICs[7,9,12,15], consistent with the paralysis, areflexia and spinal shock seen early after SCI[16–18]. The key role of brainstem-derived 5-HT is demonstrated by the repeated finding that motoneuron excitability (PICs) and associated motor functions (locomotion) can be regained shortly after SCI with exogenous application of 5-HT or selective agonists that activate 5-HT_2 receptors[7,9–11,19].

Remarkably, over the weeks after SCI (chronic injury), motoneurons spontaneously recover their excitability, with large PICs and associated sustained firing[12,20], despite the continued absence of brainstem-derived 5-HT. However, unlike before injury, the powerful depolarizing actions of PICs are difficult to terminate, because after injury motoneurons have weaker inhibitory inputs[21], especially from spinal interneurons that are normally regulated by descending tracts[12,17,22–26]. Thus, the PICs (especially Ca^{2+} PICs) can lead to excessive motoneuron activity that produces uncontrolled and debilitating muscle contractions (spasms, lasting many seconds), in both humans[27] and rats[12,17]. To make matters worse, these PICs and spasms are readily triggered by synaptic inputs arising from normally innocuous cutaneous stimulation or muscle stretch, because these synaptic inputs are enhanced after SCI[12,17,23,28–30].

A major question that remains is how motoneurons adapt so profoundly, recovering large PICs in the absence of brainstem-derived 5-HT. Here we consider the hypothesis that 5-HT_2 receptors on spinal motoneurons become constitutively active to compensate for lost brainstem 5-HT, ultimately helping to produce recovery of motoneuron excitability (PICs) and related motor functions such as locomotion. Constitutively active receptors spontaneously couple to

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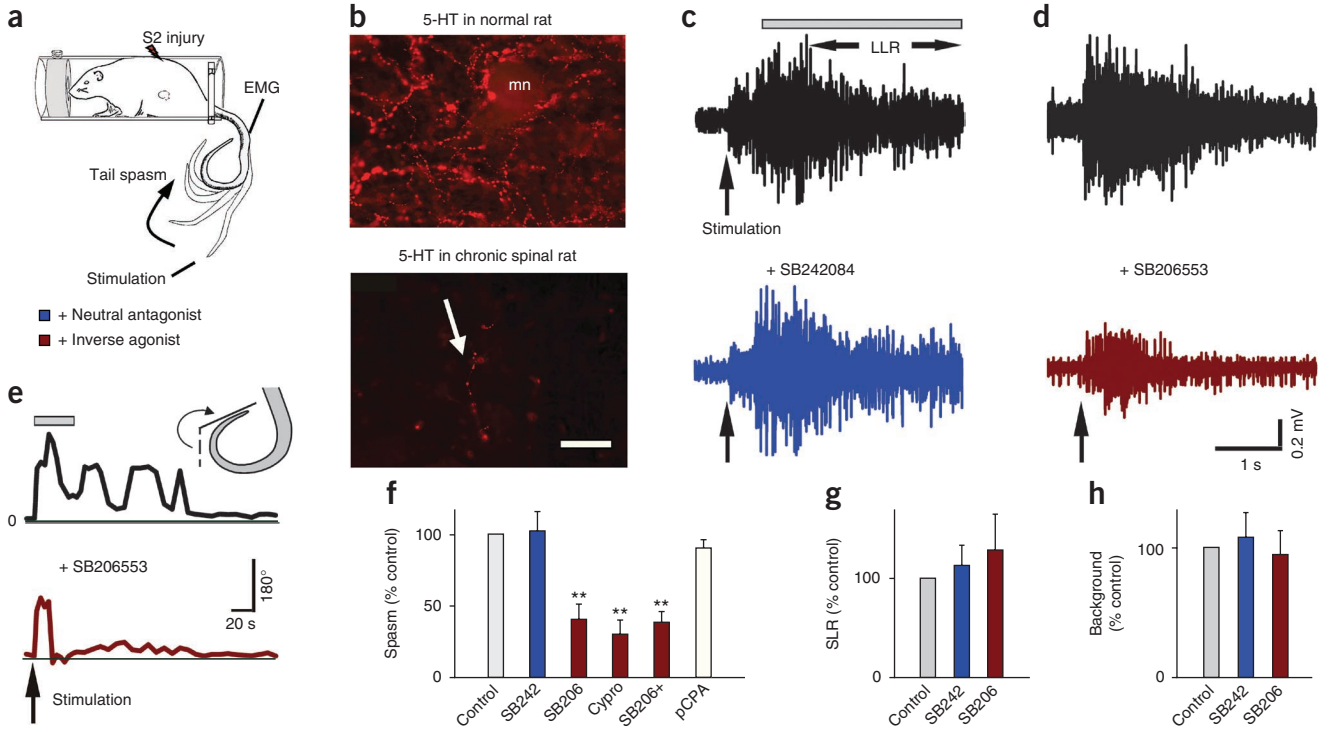

Figure 1 Constitutive 5-HT$_2$ receptor activity, but not residual 5-HT, causes spasms. (**a**) Schematic of tail spasm in an awake chronic spinal rat with S2 sacral transection. (**b**) Representative immunofluorescence images of 5-HT fibers (beaded) in the S4 ventral horn of normal rats (top; mn, motoneuron, $n = 5$ rats) and chronic spinal rats (bottom; the arrow indicates a residual fiber, $n = 5$; scale bar, 50 μm). (**c**,**d**) Spasms in chronic spinal rat evoked by cutaneous electrical stimulation of the tail (pulse three times the threshold (3×T)) and recorded with EMG (quantified during the length of time indicated by the bar, LLR) before and after blocking effects of residual 5-HT with i.t. injection of the neutral antagonist SB242084 (3 mM in 30 μl saline). (**d**) Lack of spasm (LLR) after blocking constitutive receptor activity with the inverse agonist SB206553 (i.t., 3 mM in 30 μl saline). (**e**) Tail flexion angle during spasms before and after SB206553 injection, quantified during the length of time indicated by the bar. (**f**) Group means of spasms (normalized to predrug control) with SB242084 (abbreviated SB242; LLR), SB206553 (SB206 for LLR EMG recording; and SB206+ for tail-angle spasms) and cyproheptadine (cypro; LLR; 10 mg per kg body weight, orally), and after depletion of residual 5-HT with para-chlorophenylalanine-methyl-ester (pCPA) (two 300 mg per kg body weight intraperitoneal injections over 48 h; tail-angle), with $n = 5$ rats per drug. (**g**,**h**) Normalized group means of SLR and background EMG with SB242084 and SB206553. $^{**}P < 0.01$ relative to predrug control, 100%. Error bars indicate s.e.m.

their Gq proteins and initiate intracellular signaling without being bound to 5-HT or any other ligand[31–37], a process well understood in isolated cell culture systems but not previously considered for motoneurons. The 5-HT$_{2C}$ receptor is an ideal candidate for such constitutive activity because it has a number of native isoforms that have a high degree of constitutive activity in humans and rats (>50% active)[32,35]. Furthermore, expression of these constitutively active isoforms increases in the cortex after depletion of 5-HT[38], suggesting that a similar change may be possible after SCI. We thus examined whether recovery of motoneuron function after SCI depends on constitutive 5-HT receptor activity. We initially focused on showing that constitutive receptor activity causes spasms, because the emergence of spasms after SCI is an indirect measure of recovery of motoneuron and general motor function (albeit maladaptive) that is readily studied in rats and humans (motoneuron PICs cause spasms). After this, we evaluated how constitutive activity contributes to locomotor recovery. For studying spasms, we used a complete spinal transection model (chronic spinal rat, **Fig. 1**), which eliminates brainstem-derived 5-HT, thus minimizing the chance that receptors remain activated by 5-HT. Nevertheless, we still had to consider the role of other 5-HT sources, because even with a complete transection, some residual spinal 5-HT remains caudal to the injury[39], and motoneurons are extremely sensitive to small amounts of 5-HT after SCI[8,10].

RESULTS

Lack of contribution of residual 5-HT to spasms

Before injury, the spinal cord was densely innervated by 5-HT fibers along its whole length, particularly in the ventral horn (**Fig. 1b**). In contrast, after SCI in the chronic spinal rat, only a few short (43.3 ± 25.0 μm) fibers remained (**Fig. 1b**) with, on average, 18 ± 11 such fibers along the whole length of the spinal cord below the injury.

To examine whether the remaining 5-HT fibers in chronic spinal rats had any functional effect on spasms and associated 5-HT$_2$ receptors, we blocked the action of 5-HT with an intrathecal (i.t.) injection of the highly selective 5-HT$_{2C}$ receptor antagonist SB242084 (**Fig. 1c**,**f**). This injection did not significantly change the tail muscle spasms recorded with EMG *in vivo* (**Fig. 1c**; evoked by cutaneous stimulation), indicating that the 5-HT$_2$ receptors were not activated by residual 5-HT (or other endogenous ligands). Notably, SB242084 is a neutral antagonist that blocks only the action of 5-HT (or other agonists) on the 5-HT$_2$ receptors, and does not inhibit constitutive receptor activity[31,34]. We also found that depleting residual 5-HT with pCPA[38] did not significantly influence spasms (**Fig. 1f**).

Spasms depend on constitutive 5-HT$_2$ receptor activity

We next examined whether the loss of 5-HT after injury was compensated for by constitutive activity in 5-HT$_2$ receptors by intrathecally injecting SB206553, which selectively binds 5-HT$_{2C}$ receptors and potently

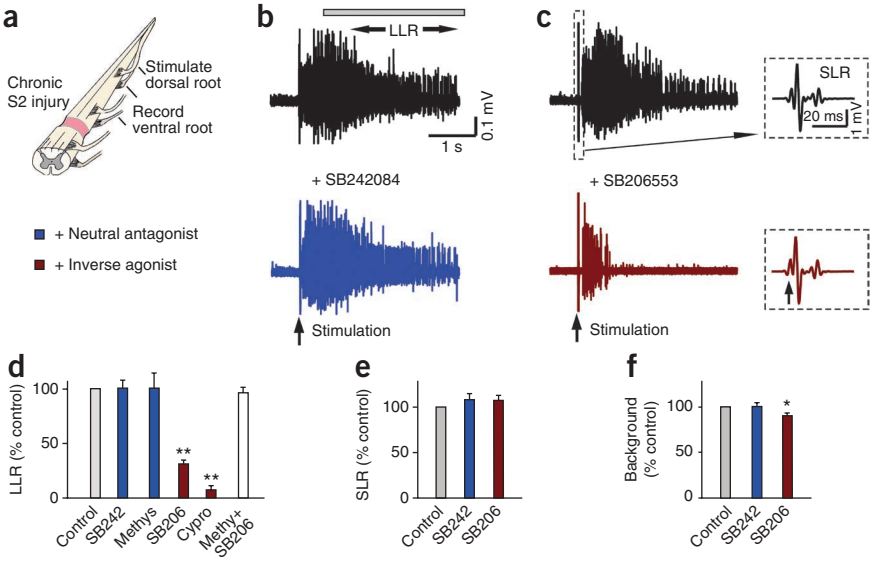

Figure 2 Constitutive 5-HT$_2$ receptor activity contributes to LLRs in the isolated spinal cord *in vitro*. (**a**) Whole sacrocaudal spinal cord below chronic S2 transection maintained *in vitro*. (**b**) Long-lasting reflex triggered by dorsal root stimulation (single pulse, 3×T) and recorded from the ventral roots (LLR, quantified during the length of time indicated by the horizontal bar; counterpart of spasms in **Figure 1**) before and after blocking effects of residual 5-HT with the neutral 5-HT$_2$ receptor antagonist SB242084 (3–5 μM). (**c**) Elimination of LLR, but not SLR, after blocking constitutive 5-HT$_2$ receptor activity with the inverse agonist SB206553 (3–5 μM). Inset, SLR (expanded time scale). (**d**) Group means of LLRs (normalized to predrug LLRs) with SB242084 (abbreviated SB242, $n = 11$), methysergide (Methys, 10 μM, neutral antagonist, $n = 12$), SB206553 (SB206, $n = 24$), cyproheptadine (Cypro, 20 μM; $n = 6$), and SB206553 after prior application of methysergide (30 μM; white bar; Methy+SB206; $n = 8$). (**e**,**f**) Normalized group means of the SLR and background ventral root activity with SB206553 and SB242084. $^*P < 0.05$, $^{**}P < 0.01$ relative to control, 100%. Error bars indicate s.e.m.

inhibits their constitutive activity (termed an inverse agonist[31,34,37]). This injection reduced the magnitude of the spasms recorded with either electromyography (EMG) (**Fig. 1d**,**f**) or tail kinematics (**Fig. 1e**,**f**) by well over 50%, whereas control saline injections had no effect. Likewise, oral application of the non-selective 5-HT$_2$ receptor inverse agonist cyproheptadine[33] significantly reduced spasms (**Fig. 1f**).

We next examined the whole spinal cord from chronic spinal rats (caudal to the injury) after it was removed and maintained *in vitro*, which eliminated possible peripheral or brain-derived 5-HT influences. We recorded long-lasting reflexes (LLRs) from the ventral roots in response to a brief stimulation of dorsal roots (**Fig. 2a–c**); these LLRs have previously been shown to underlie muscle spasms recorded *in vivo*[12,17]. The LLRs were not significantly affected by blocking the possible action of endogenous 5-HT with the 5-HT$_{2C}$ receptor neutral antagonists SB242084 or methysergide[33] (**Fig. 2b**,**d**), even though these antagonists blocked the increase in LLRs induced by exogenous application of selective 5-HT$_{2C}$ agonists (**Supplementary Fig. 1**). Furthermore, enhancing available residual endogenous 5-HT with either the 5-HT transport-blocker citalopram or 5-HT releaser fenfluramine did not significantly affect LLRs (**Supplementary Fig. 2**). In contrast, the LLRs were markedly inhibited by blocking constitutive 5-HT$_2$ receptor activity with inverse agonists (SB206553 and cyproheptadine; **Fig. 2c**,**d**). This inhibitory action of SB206553 was blocked by a prior application of methysergide (**Fig. 2d**), which competitively inhibits SB206553 binding to 5-HT$_{2C}$ receptors[34].

The transient short latency reflexes (SLRs) evoked immediately after stimulation (**Fig. 2c**) were not affected by SB206553, both *in vitro* (**Fig. 2e**) and *in vivo* (**Fig. 1g**), and did not correlate with the LLRs (spasms; $r^2 = 0.10$)[29], consistent with a negligible modulation of SLRs by Ca^{2+} PICs and associated 5-HT$_{2C}$ receptors. Also, the background activity before the LLRs had relatively little (**Fig. 2f**, *in vitro*) or no (**Fig. 1h**, *in vivo*) change with SB206553 treatment.

Constitutive 5-HT$_2$ receptor activity in motoneurons

Given that spasms result from persistent calcium currents (Ca^{2+} PICs) in motoneurons[12,27], we made intracellular recordings from motoneurons after SCI to investigate whether there were constitutively active 5-HT$_{2C}$ receptors on motoneurons that regulate Ca^{2+} PICs (**Fig. 3**). As previously described, the large voltage-dependent Ca^{2+} PICs in motoneurons were readily observed in isolation as a sharp downward deflection in the current response during an increasing voltage ramp (**Fig. 3b**) after sodium currents and synaptic inputs were eliminated with tetrodotoxin[12]. Blocking constitutively active 5-HT$_2$ receptors with the inverse agonists SB206553 or cyproheptadine markedly decreased the magnitude of these Ca^{2+} PICs (**Fig. 3b**,**f**), whereas SB242084 had no effect on Ca^{2+} PICs (**Fig. 3c**,**f**). The portion of the Ca^{2+} PICs that resulted from constitutive 5-HT$_2$ receptor activity (SB206553-sensitive decrease) was 1.99 ± 0.42 nA, which was 42.9 ± 8.9% of the maximum possible Ca^{2+} PICs produced by activating all 5-HT$_2$ receptors (with 1 μM 5-HT). The small remaining Ca^{2+} PICs with inverse agonists in chronic spinal rats was similar to the small Ca^{2+} PICs observed acutely after spinal transection (**Fig. 3f**).

When we stimulated the dorsal roots during recording from a motoneuron at rest and in the absence of tetrodotoxin, the PIC produced a sustained depolarization (plateau)[12] that caused many seconds of repetitive firing (LLR; **Fig. 3d**). As expected, the LLR and plateau were eliminated by the inverse agonist SB206553 (**Fig. 3e**). The LLR and plateau were also eliminated by simply hyperpolarizing the motoneuron to prevent activation of the underlying voltage-dependent PIC (**Fig. 3d**)[12], although there remained a polysynaptic excitatory postsynaptic potential (EPSP) lasting about 0.5 s. The inverse agonists SB206553 and cyproheptadine had no effect on this EPSP (**Fig. 3e**,**g**).

Increase in constitutively active 5-HT$_{2C}$ receptor isoform

The 5-HT$_{2C}$ receptor RNA undergoes post-transcriptional editing at five sites (labeled A to E) that leads to numerous native receptor isoforms in rats and humans, by changing three amino acids on an intracellular loop of the receptor (isoforms are named after the amino acid sequences, such as INI, VSV and VNI, as depicted in **Fig. 4a**)[32–35,40]. Functionally, the unedited isoform (INI) shows a high degree of constitutive activity, whereas editing reduces this activity, producing isoforms with less constitutive activity, such as

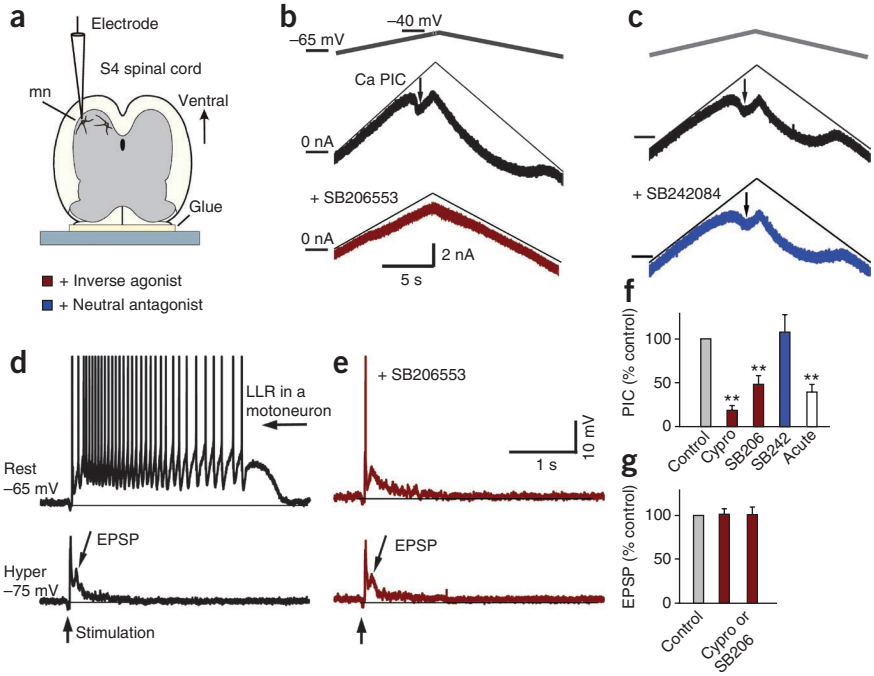

Figure 3 Constitutively active 5-HT$_2$ receptors on motoneurons contribute to Ca^{2+} PICs underlying spasms. (**a**,**b**) Intracellular recording from motoneuron (mn) in whole spinal cord, *in vitro*. (**b**) Top, Ca^{2+} PIC in motoneuron of chronic spinal rat, activated by slowly increasing the membrane potential under voltage-clamp in presence of 2 μM tetrodotoxin (TTX) and quantified at its initial peak, where it produced a downward deflection in the recorded current (thick black plot, at arrow, Ca^{2+} PIC) relative to the leak current (thin line). Bottom plot, small Ca^{2+} PIC after SB206553 application (5 μM). (**c**) Ca^{2+} PIC in another motoneuron (arrow), which is unaffected by SB242084 application (5 μM). (**d**) Top, PIC-mediated plateau and sustained firing (LLR) evoked by dorsal root stimulation (3×T; without TTX) in a motoneuron at rest (without injected current; top). Bottom, with a hyperpolarizing bias current to prevent PIC activation, the same stimulation only evoked a polysynaptic EPSP (lower plot). (**e**) Response of same motoneuron as in **d** to dorsal root stimulation after application of SB206553 (5 μM), at rest (top) and with a hyperpolarizing bias current (bottom). (**f**) Group means of Ca^{2+} PIC (normalized to predrug Ca^{2+} PIC in chronic spinal rats, control), with SB206553 (SB206; $n = 7$), cyproheptadine (cypro, 20 μM; $n = 16$) and SB242084 (SB242; $n = 5$) in chronic spinal rats and in acute spinal rats (white bar, no drugs, $n = 7$). (**g**) Normalized group means of EPSP amplitude (middle bar; control mean 4.4 mV) and duration (right bar, control 480 ms) with inverse agonists cyproheptadine or SB206553 (chronic). $^{**}P < 0.01$ relative to control, 100%. Error bars represent s.e.m.

VNI (with 51% of INI activity) and VSV (32% of INI)[36]. We thus compared 5-HT$_{2C}$ receptor mRNA levels from spinal cords of normal (unlesioned) and chronic spinal rats (below S2 injury level). The total amount of 5-HT$_{2C}$ mRNA did not change with SCI (**Fig. 4b**). However, there was a decrease in the amount of RNA editing at the A site (**Fig. 4c**). Corresponding to this, there was also a decrease in the relative proportion of the VNI receptor isoform and an increase in the relative proportion of the highly constitutively active INI isoform (**Fig. 4d**). The increase in INI isoform expression (400%) was similar to the increase in PIC with chronic injury (**Fig. 4e**).

We directly confirmed that the motoneurons of the sacral spinal cord had 5-HT$_{2C}$ receptors after SCI by immunolabeling (**Supplementary Fig. 3**). Furthermore, a large fraction of the 5-HT$_{2C}$ receptor labeling was inside the motoneurons (intracellular) in chronic spinal rats, and this receptor internalization was reduced by SB206553, consistent with the presence of constitutively active isoforms of the receptor on motoneurons, the hallmark of which is a high degree of activity-dependent internalization (INI isoform[34,37]; **Supplementary Figs. 3** and **4**).

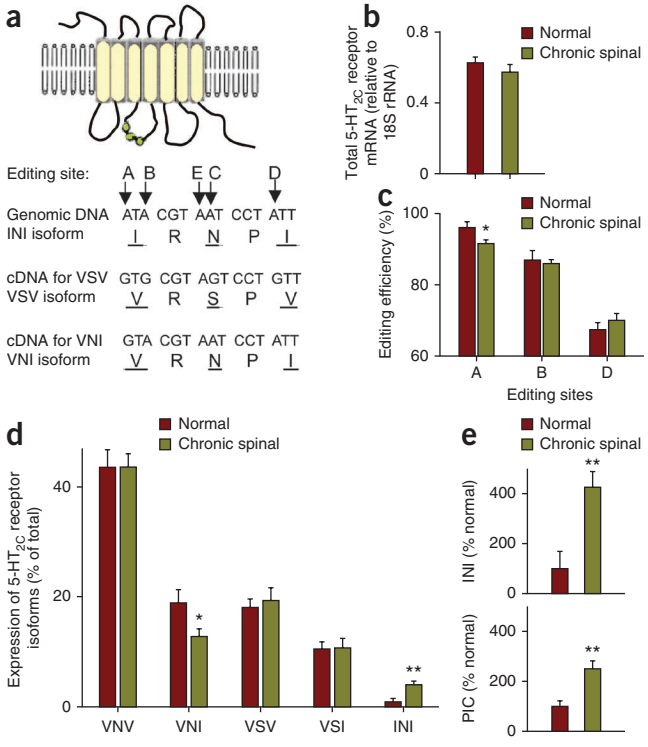

Figure 4 A highly constitutively active 5-HT$_{2C}$ receptor isoform is upregulated with injury. (**a**) Schematic showing 5-HT$_{2C}$ receptor with various isoforms produced by changing three amino acids on its intracellular loop (green; isoforms named by amino acid triplet). These three amino acids (underlined) are changed by post-transcriptional editing of RNA at five sites (A–E; adenosine editing), leading to various native receptor isoforms, of which the unedited isoform (INI) is most highly constitutively active. (**b**) Total 5-HT$_{2C}$ receptor mRNA (normalized to an internal control, 18S rRNA) in chronic spinal rats ($n = 6$) and normal uninjured rats ($n = 6$). (**c**) Proportion of 5-HT$_{2C}$ receptor mRNA with editing at sites A, B and D (editing efficiency) in chronic spinal and normal rats (C and E site editing efficiency < 30% and not changed, data not shown). (**d**) Distribution of 5-HT$_{2C}$ receptor isoform mRNA in the spinal cord of normal and chronic spinal rats (15 isoforms detected; the five most prevalent are shown). (**e**) Comparison of change in INI isoform expression (top) and Ca^{2+} PIC (bottom, recorded *in vitro*) after chronic spinal injury. $^{*}P < 0.05$, $^{**}P < 0.01$, significant change with injury. Error bars indicate s.e.m.

Antispastic action of inverse agonists in humans with SCI

In humans with SCI, we evoked leg muscle spasms with cutaneous stimulation of the foot while recording tibialis anterior muscle EMG

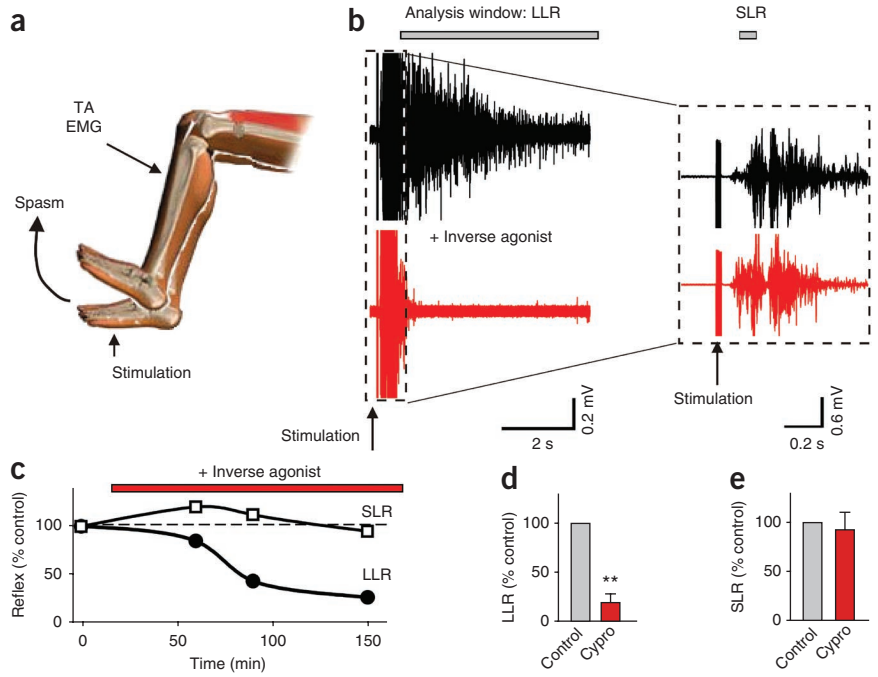

Figure 5 5-HT$_2$ receptor inverse agonist blocks spasms in spinal cord injured humans. (**a**) Leg spasm triggered by brief electrical stimulation of the medial arch of the foot (3–5×T). TA, tibialis anterior. (**b**) Spasm recorded with tibialis anterior muscle surface EMG and quantified over the time windows indicated (LLR and SLR), before and 2 h after blocking constitutively active 5-HT$_2$ receptors with cyproheptadine (8 mg administered orally). The inset on a different scale shows SLR. (**c**) Gradual reduction in the spasms (LLRs), but not SLRs, over time after inverse agonist application. (**d**,**e**) Normalized group means for LLRs (**d**) and SLRs (**e**) with cyproheptadine ($n = 7$ subjects). $^{**}P < 0.01$ relative to control, 100%. Error bars represent s.e.m.

(**Fig. 5**)[27]. Blocking constitutive 5-HT$_2$ receptor activity with oral administration of the inverse agonist cyproheptadine significantly decreased the muscle spasms (**Fig. 5b**,**d**). Furthermore, the effect was again selective to the long-lasting portion of the spasm (LLR, **Fig. 5b–d**), with no drug-induced change in the SLR (**Fig. 5b**,**e**). Spasms were equally reduced by cyproheptadine in subjects with varying impairment of motor function (B–D on the American Spinal Injury Association Impairment Scale, which ranges from A–E; **Supplementary Table 1**).

Dependence of walking on constitutively active 5-HT$_2$ receptors

To evaluate whether constitutive 5-HT receptor activity contributes to recovery of locomotion after partial SCI, we used a staggered hemisection injury model (**Fig. 6a**) that transects all descending 5-HT axons but spares enough propriospinal neurons that traverse the injury site to allow the rat to voluntarily initiate functional hindlimb locomotion[41]. Three weeks after this injury, rats regained good hindlimb locomotor ability, voluntarily initiating walking with near normal weight support, although they retained a deficit in forelimb-hindlimb coordination (with a BBB score[42] < 12; **Fig. 6b**). Blocking constitutively active 5-HT$_2$ receptors with the inverse agonist SB206553 (i.t.) dramatically reduced weight support (hindlimbs dragged; **Fig. 6c**) and overall locomotor ability (BBB score, **Fig. 6c**,**d**). In contrast, blocking possible action of residual 5-HT with the neutral antagonist SB242084 had no significant effect (**Fig. 6d**).

DISCUSSION

A loss of brainstem-derived 5-HT after SCI acutely reduces motoneuron excitability[6–9,15] and accordingly depresses all motor functions. Our results demonstrate a previously undescribed mechanism for how spinal motoneurons compensate for this lost 5-HT over the months after injury (chronic injury). Decreased editing at a single site on the 5-HT$_{2C}$ receptor RNA (A site) leads to increased expression of the constitutively active INI isoform of this receptor. Constitutive 5-HT$_2$ receptor activity in turn leads to large Ca^{2+} PICs in motoneurons, which ultimately enable motoneurons to recover their excitability, as evidenced by their sensitivity to inverse agonists. Because large PICs in motoneurons have been shown to have key roles in normal motor function in uninjured humans and animals[11], these results suggest that constitutive 5-HT receptor activity (with its associated PICs) is essential in recovery of motor function after SCI. Indeed, we show

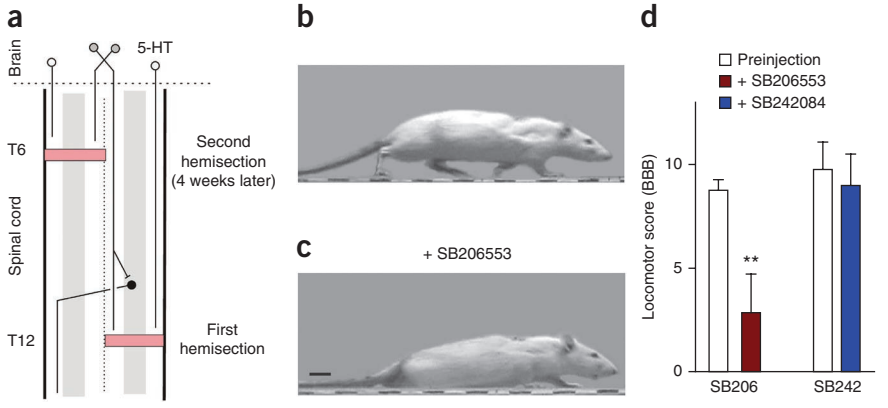

Figure 6 Spontaneous recovery of locomotion in staggered-hemisected rats depends on constitutively active 5-HT$_2$ receptors. (**a**) Schematic of staggered-hemisection SCI, which transects all descending axons from the brain, including 5-HT neurons (white circles), but leaves local propriospinal neurons (black) that transverse the injury and help relay descending signals for initiation of locomotion (gray)[41]. (**b**) Rat walking with good weight support and toe clearance three weeks after the staggered-hemisection (after second hemisection). (**c**) Same rat with little hindlimb weight support (just foot paddling motions), while the forelimbs dragged the hindquarters during walking after blocking constitutively active 5-HT$_2$ receptors with SB206553 (3 mM in 30 µl saline, i.t.; same dose as in **Fig. 1**). Scale bar, 2 cm. (**d**) Group means of BBB locomotor scores before and after SB206553 injection ($n = 8$) and control SB242084 injection (3 mM in 30 µl saline, i.t.; $n = 8$ rats). $^{**}P < 0.01$ relative to preinjection. Error bars represent s.e.m.

that constitutive 5-HT_2 receptor activity is crucial for spontaneous recovery of hindlimb locomotor function after partial SCI, because inverse agonists impair locomotion.

Given that inverse agonists inhibit conventional activation of 5-HT_2 receptors by 5-HT, as well as constitutive activity, their action alone is not definitive proof of constitutive activity, without ruling out the influence of endogenous residual 5-HT[31,34]. We thus ruled out residual 5-HT by showing a complete lack of effect of neutral antagonists, 5-HT depletion, *in vitro* spinal cord isolation, SERT blockers and 5-HT releasers after SCI in rats.

Our results also show that, without normal descending supraspinal control, these constitutively active 5-HT_2 receptors and associated PICs can, unfortunately, lead to uncontrolled motoneuron firing and associated muscle spasms (LLRs), which emerge over the weeks after injury[17]. However, blocking this constitutive receptor activity with inverse agonists decreases spasms in rats and humans with SCI, suggesting a new rationale for antispastic drug development, although care must be taken to use a dose that preserves some residual function. For example, the high dose of SB206553 used here to maximally block spasms in the transected rat also eliminates locomotion in the rat after partial SCI. In contrast, low doses of the broad-spectrum inverse agonist cyproheptadine have been shown to improve locomotion in humans[43], presumably by reducing the amplitude and incidence[44] of spasms that can interfere with stepping without completely eliminating PICs and muscle strength. The EPSPs that trigger spasms (and associated SLRs) are not affected by 5-HT_{2C} receptor inverse agonists, whereas they are inhibited by traditional antispastic drugs such as baclofen, because they are regulated by other receptors presynaptically[45,46]. Thus, inverse agonists provide an independent and complementary approach to traditional spasticity management[29,30,46].

Taken together, our pharmacological, mRNA and immunolabeling data suggest that the large PICs on motoneurons after SCI are facilitated by constitutive activity in 5-HT_{2C} type receptors on motoneurons (perhaps with additional involvement of 5-HT_{2B} receptors, because SB206553 blocks both 5-HT_{2B} and 5-HT_{2C} receptors[47–49]). 5-HT_{2C} receptors activate the intracellular phospholipase C (PLC) pathway that leads to inositol phosphate synthesis and mobilization of intracellular Ca^{2+} stores[50,51]. Constitutive 5-HT_{2C} receptor activity leads to a basal level of activity in this PLC pathway, which is inhibited by receptor blockade with inverse agonists such as SB206553 but not by neutral antagonists such as SB242084 (refs. 31,34,47,48). Our analogous results with SB206553 and SB242084 suggest that an intracellular PLC pathway in motoneurons may be tonically activated after SCI by constitutive activity, especially considering that motoneurons (PICs) are known to be regulated by PLC, inositol phosphate and intracellular Ca^{2+} concentrations[52–54].

The INI 5-HT_{2C} receptor isoform that we find upregulated in the spinal cord after chronic SCI shows substantial constitutive activity, with basal levels of inositol phosphate production approaching that achieved by 5-HT (fully active)[32,35]. Other isoforms show substantially less constitutive activity[32,36] and do not increase in expression with injury. However, these isoforms probably contribute to a basal level of constitutive receptor activity in the normal rat, which should persist acutely after injury, contributing to the small PICs measured *in vitro* in the acutely isolated spinal cord of normal rats[15]. Also, the increase in total 5-HT_{2C} receptor expression reported with severe chronic SCI[55] should increase the constitutive activity contributed from all isoforms. This might explain why the PIC that is produced by constitutive activity (SB206553-sensitive) after chronic SCI is about 40% of the maximum PIC that can be induced by activating all 5-HT_2 receptors, even though INI isoform represents only about 4% of all 5-HT_{2C} receptors after injury. This discrepancy might also be explained by the especially effective intracellular signaling capacity of INI receptor isoforms, producing many times more inositol phosphate than other isoform[56], and thus perhaps producing a disproportionately large PIC.

We do not know what initiates the remarkable adaptation in 5-HT_{2C} receptors that we see after SCI. Perhaps it is the loss of 5-HT itself[38]. Alternatively, the lack of motoneuron activity and associated intracellular calcium signaling may trigger the adaptation, as in synaptically isolated single neurons[57]. That is, motoneurons may require an optimal amount of activity, regardless of where it arises or what form it takes (spasms or walking), and activity-dependent tuning of constitutive activity in 5-HT receptors may help achieve such optimal activity. Perhaps this explains why intense locomotor training activity after SCI in humans not only improves walking but also reduces spastic muscle activity[58].

Our finding of constitutive 5-HT receptor activity opens up new possibilities for understanding spinal cord plasticity in disease and injury. Although the spinal cord is densely innervated by brainstem-derived 5-HT fibers, there are actually relatively few neurons in the brainstem that provide all of this innervation (<10,000; each neuron branches extensively)[4], leaving motoneurons and spinal functions vulnerable to injury or disease that affects activity in these few 5-HT neurons. Constitutive 5-HT_2 receptor activity provides a safeguard against such loss of 5-HT innervation of the spinal cord and probably even contributes to basal receptor activity in normal rats. With the loss of 5-HT after SCI, this constitutive activity increases dramatically, replacing the lost 5-HT–mediated activity.

In summary, we have demonstrated that substantial constitutive 5-HT_{2C} receptor activity emerges after SCI and contributes to recovery of motoneuron function, with both positive (walking) and negative (spasms) outcomes. This constitutive activity must work in concert with the many other factors that contribute to locomotion and spasticity[5,22,23,28–30,41,45,59].

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

K.C.M. performed the *in vitro* rat experiments, contributed to all other rat studies and co-wrote the paper. M.R., P.J.H., R.L., W.H., L.S., M.J.S., R.V., X.L. and K.F. contributed to the *in vivo* rat experiments. K.F., R.V., E.W.B., R.A. and C.J.H. contributed to immunolabeling experiments. K.F. co-wrote the paper and shared equally with D.J.B. in senior authorship (last author). A.N. and T.M. conducted mRNA analysis. J.D. and M.A.G. conducted the human experiments. D.J.B. performed *in vitro* and *in vivo* rat experiments, supervised or co-supervised all of the experiments and co-wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Rekling, J.C., Funk, G.D., Bayliss, D.A., Dong, X.W. & Feldman, J.L. Synaptic control of motoneuronal excitability. *Physiol. Rev.* **80**, 767–852 (2000).
2. Hultborn, H., Denton, M.E., Wienecke, J. & Nielsen, J.B. Variable amplification of synaptic input to cat spinal motoneurones by dendritic persistent inward current. *J. Physiol. (Lond.)* **552**, 945–952 (2003).
3. Carlsson, A., Magnusson, T. & Rosengren, E. 5-hydroxytryptamine of the spinal cord normally and after transection. *Experientia* **19**, 359 (1963).
4. Jacobs, B.L., Martin-Cora, F.J. & Fornal, C.A. Activity of medullary serotonergic neurons in freely moving animals. *Brain Res. Brain Res. Rev.* **40**, 45–52 (2002).
5. Jordan, L.M., Liu, J., Hedlund, P.B., Akay, T. & Pearson, K.G. Descending command systems for the initiation of locomotion in mammals. *Brain Res. Rev.* **57**, 183–191 (2008).
6. Perrier, J.F. & Delgado-Lezama, R. Synaptic release of serotonin induced by stimulation of the raphe nucleus promotes plateau potentials in spinal motoneurons of the adult turtle. *J. Neurosci.* **25**, 7993–7999 (2005).
7. Hounsgaard, J., Hultborn, H., Jespersen, B. & Kiehn, O. Bistability of α-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J. Physiol. (Lond.)* **405**, 345–367 (1988).
8. Li, X., Murray, K., Harvey, P.J., Ballou, E.W. & Bennett, D.J. Serotonin facilitates a persistent calcium current in motoneurons of rats with and without chronic spinal cord injury. *J. Neurophysiol.* **97**, 1236–1246 (2007).
9. Perrier, J.F. & Hounsgaard, J. 5–HT2 receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. *J. Neurophysiol.* **89**, 954–959 (2003).
10. Harvey, P.J., Li, X., Li, Y. & Bennett, D.J. 5–HT2 receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *J. Neurophysiol.* **96**, 1158–1170 (2006).
11. Heckmann, C.J., Gorassini, M.A. & Bennett, D.J. Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve* **31**, 135–156 (2005).
12. Li, Y., Gorassini, M.A. & Bennett, D.J. Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. *J. Neurophysiol.* **91**, 767–783 (2004).
13. Carlin, K.P., Jiang, Z. & Brownstone, R.M. Characterization of calcium currents in functionally mature mouse spinal motoneurons. *Eur. J. Neurosci.* **12**, 1624–1634 (2000).
14. Brownstone, R.M., Gossard, J.P. & Hultborn, H. Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. *Exp. Brain Res.* **102**, 34–44 (1994).
15. Harvey, P.J., Li, Y., Li, X. & Bennett, D.J. Persistent sodium currents and repetitive firing in motoneurons of the sacrocaudal spinal cord of adult rats. *J. Neurophysiol.* **96**, 1141–1157 (2006).
16. Bennett, D.J. *et al.* Spasticity in rats with sacral spinal cord injury. *J. Neurotrauma* **16**, 69–84 (1999).
17. Bennett, D.J., Sanelli, L., Cooke, C., Harvey, P.J. & Gorassini, M.A. Spastic long-lasting reflexes in the awake rat after sacral spinal cord injury. *J. Neurophysiol.* **91**, 2247–2258 (2004).
18. Kuhn, R.A. & Macht, M.B. Some manifestations of reflex activity in spinal man with particular reference to the occurrence of extensor spasm. *Bull. Johns Hopkins Hosp.* **84**, 43–75 (1949).
19. Ung, R.V. *et al.* Role of spinal 5–HT2 receptor subtypes in quipazine-induced hindlimb movements after a low-thoracic spinal cord transection. *Eur. J. Neurosci.* **28**, 2231–2242 (2008).
20. Button, D.C. *et al.* Does elimination of afferent input modify the changes in rat motoneurone properties that occur following chronic spinal cord transection? *J. Physiol. (Lond.)* **586**, 529–544 (2008).
21. Boulenguez, P. *et al.* Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat. Med.* **16**, 302–307 (2010).
22. Crone, C., Johnsen, L.L., Biering-Sorensen, F. & Nielsen, J.B. Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury. *Brain* **126**, 495–507 (2003).
23. Norton, J.A., Bennett, D.J., Knash, M.E., Murray, K.C. & Gorassini, M.A. Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. *Brain* **131**, 1478–1491 (2008).
24. Baldissera, F., Hultborn, H. & Illert, M. Integration in spinal neuronal systems. in *Handbook of Physiology. The Nervous System. Motor Control* (ed. Brooks, V.B.) 509–595 (American Physiological Society, Bethesda, Maryland, 1981).
25. Shefchyk, S.J. & Jordan, L.M. Excitatory and inhibitory postsynaptic potentials in alpha-motoneurons produced during fictive locomotion by stimulation of the mesencephalic locomotor region. *J. Neurophysiol.* **53**, 1345–1355 (1985).
26. Wallis, D.I., Wu, J. & Wang, X. Descending inhibition in the neonate rat spinal cord is mediated by 5-hydroxytryptamine. *Neuropharmacology* **32**, 73–83 (1993).
27. Gorassini, M.A., Knash, M.E., Harvey, P.J., Bennett, D.J. & Yang, J.F. Role of motoneurons in the generation of muscle spasms after spinal cord injury. *Brain* **127**, 2247–2258 (2004).
28. Baker, L.L. & Chandler, S.H. Characterization of postsynaptic potentials evoked by sural nerve stimulation in hindlimb motoneurons from acute and chronic spinal cats. *Brain Res.* **420**, 340–350 (1987).
29. Dietz, V. & Sinkjaer, T. Spastic movement disorder: impaired reflex function and altered muscle mechanics. *Lancet Neurol.* **6**, 725–733 (2007).
30. Ashby, P. & McCrea, D.A. Neurphysiology of spinal spasticity. in *Handbook of the Spinal Cord* (ed. Davidoff, R.A.) 119–143 (Dekker, New York, 1987).
31. Seifert, R. & Wenzel-Seifert, K. Constitutive activity of G protein–coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **366**, 381–416 (2002).
32. Herrick-Davis, K., Grinde, E. & Niswender, C.M. Serotonin 5–HT2C receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. *J. Neurochem.* **73**, 1711–1717 (1999).
33. Westphal, R.S. & Sanders-Bush, E. Reciprocal binding properties of 5-hydroxytryptamine type 2C receptor agonists and inverse agonists. *Mol. Pharmacol.* **46**, 937–942 (1994).
34. Chanrion, B. *et al.* Inverse agonist and neutral antagonist actions of antidepressants at recombinant and native 5-hydroxytryptamine2C receptors: differential modulation of cell surface expression and signal transduction. *Mol. Pharmacol.* **73**, 748–757 (2008).
35. Niswender, C.M., Copeland, S.C., Herrick-Davis, K., Emeson, R.B. & Sanders-Bush, E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.* **274**, 9472–9478 (1999).
36. Berg, K.A., Dunlop, J., Sanchez, T., Silva, M. & Clarke, W.P. A conservative, single-amino acid substitution in the second cytoplasmic domain of the human Serotonin2C receptor alters both ligand-dependent and -independent receptor signaling. *J. Pharmacol. Exp. Ther.* **324**, 1084–1092 (2008).
37. Marion, S., Weiner, D.M. & Caron, M.G. RNA editing induces variation in desensitization and trafficking of 5-hydroxytryptamine 2c receptor isoforms. *J. Biol. Chem.* **279**, 2945–2954 (2004).
38. Gurevich, I., Englander, M.T., Adlersberg, M., Siegal, N.B. & Schmauss, C. Modulation of serotonin 2C receptor editing by sustained changes in serotonergic neurotransmission. *J. Neurosci.* **22**, 10529–10532 (2002).
39. Newton, B.W. & Hamill, R.W. The morphology and distribution of rat serotoninergic intraspinal neurons: an immunohistochemical study. *Brain Res. Bull.* **20**, 349–360 (1988).
40. Nakae, A. *et al.* Serotonin2C receptor mRNA editing in neuropathic pain model. *Neurosci. Res.* **60**, 228–231 (2008).
41. Courtine, G. *et al.* Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nat. Med.* **14**, 69–74 (2008).
42. Basso, D.M., Beattie, M.S. & Bresnahan, J.C. A sensitive and reliable locomotor rating scale for open field testing in rats. *J. Neurotrauma* **12**, 1–21 (1995).
43. Wainberg, M., Barbeau, H. & Gauthier, S. The effects of cyproheptadine on locomotion and on spasticity in patients with spinal cord injuries. *J. Neurol. Neurosurg. Psychiatry* **53**, 754–763 (1990).
44. Barbeau, H., Richards, C.L. & Bedard, P.J. Action of cyproheptadine in spastic paraparetic patients. *J. Neurol. Neurosurg. Psychiatry* **45**, 923–926 (1982).
45. Li, Y., Li, X., Harvey, P.J. & Bennett, D.J. Effects of baclofen on spinal reflexes and persistent inward currents in motoneurons of chronic spinal rats with spasticity. *J. Neurophysiol.* **92**, 2694–2703 (2004).
46. Davidoff, R.A. Antispasticity drugs: mechanisms of action. *Ann. Neurol.* **17**, 107–116 (1985).
47. Kennett, G.A. *et al. In vitro* and *in vivo* profile of SB 206553, a potent 5–HT2C/5–HT2B receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.* **117**, 427–434 (1996).
48. Kennett, G.A. *et al.* SB 242084, a selective and brain penetrant 5–HT2C receptor antagonist. *Neuropharmacology* **36**, 609–620 (1997).
49. Knight, A.R. *et al.* Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **370**, 114–123 (2004).
50. Hoyer, D., Hannon, J.P. & Martin, G.R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **71**, 533–554 (2002).
51. Mizuno, N. & Itoh, H. Functions and regulatory mechanisms of Gq-signaling pathways. *Neurosignals* **17**, 42–54 (2009).
52. Mejia-Gervacio, S., Hounsgaard, J. & Diaz-Munoz, M. Roles of ryanodine and inositol triphosphate receptors in regulation of plateau potentials in turtle spinal motoneurons. *Neuroscience* **123**, 123–130 (2004).
53. Perrier, J.F., Mejia-Gervacio, S. & Hounsgaard, J. Facilitation of plateau potentials in turtle motoneurones by a pathway dependent on calcium and calmodulin. *J. Physiol. (Lond.)* **528**, 107–113 (2000).
54. Holohean, A.M. & Hackman, J.C. Mechanisms intrinsic to 5–HT2B receptor-induced potentiation of NMDA receptor responses in frog motoneurones. *Br. J. Pharmacol.* **143**, 351–360 (2004).
55. Hayashi, Y. *et al.* 5-HT precursor loading, but not 5-HT receptor agonists, increases motor function after spinal cord contusion in adult rats. *Exp. Neurol.* **221**, 68–78 (2010).
56. McGrew, L., Price, R.D., Hackler, E., Chang, M.S. & Sanders-Bush, E. RNA editing of the human serotonin 5–HT2C receptor disrupts transactivation of the small G-protein RhoA. *Mol. Pharmacol.* **65**, 252–256 (2004).
57. Turrigiano, G., Abbott, L.F. & Marder, E. Activity-dependent changes in the intrinsic properties of cultured neurons. *Science* **264**, 974–977 (1994).
58. Gorassini, M.A., Norton, J.A., Nevett-Duchcherer, J., Roy, F.D. & Yang, J.F. Changes in locomotor muscle activity after treadmill training in subjects with incomplete spinal cord injury. *J. Neurophysiol.* **101**, 969–979 (2009).
59. Rank, M.M., Li, X., Bennett, D.J. & Gorassini, M.A. Role of endogenous release of norepinephrine in muscle spasms after chronic spinal cord injury. *J. Neurophysiol.* **97**, 3166–3180 (2007).

ONLINE METHODS

Spinal lesions. All rat use was approved by the University of Alberta Animal Care and Use Committee: Health Sciences. We completely transected spinal cords of adult female Sprague-Dawley rats (locally bred) at the S2 sacral spinal level and evaluated spasticity and motoneuron properties 6–12 weeks post-injury (chronic spinal state, see **Supplementary Methods**)[12,17]. Also, a separate group of female rats underwent a staggered hemisection[41], which, like a transection, removes most descending supraspinal axons below the injury (including 5-HT axons), but leaves intact some propriospinal neuron connections that enable the rat to voluntarily initiate walking, as detailed in the **Supplementary Methods**.

All human experiments were carried out with signed, informed consent of subjects and approved by the University of Alberta Health Research Ethics Board. Human subjects had chronic SCI with varied severity (**Supplementary Table 1**) and did not take their antispastic medications on the experiment day.

Spasms in awake chronic spinal rats. We evoked tail muscle spasms with brief electrical (3× afferent threshold (T)) or manual stimulation of the skin of the tail and recorded these spasms with tail muscle EMG and video kinematic analysis, as detailed in the **Supplementary Methods**. Briefly, EMG was rectified and averaged over 10–40 ms after stimulus (SLR) and 500–4,000 ms after stimulus (LLR), and tail flexion angle measured.

Spasms in humans with SCI. We evoked leg spasms with a brief electrical stimulation of the medial arch of the foot (3–5×T) and recorded surface EMG responses over the tibialis anterior (TA) muscle (**Fig. 5**)[27]. We computed the SLR and LLR by averaging EMG over the intervals 50–100 and 500–5,000 ms after stimulation, respectively, and then subtracting background EMG (see **Supplementary Methods**).

Ventral root and intracellular motoneuron recording in rats, *in vitro*. The whole spinal cord caudal to the S2 injury level was removed from chronic spinal rats and maintained *in vitro* for ventral root and intracellular motoneuron recordings[12,45], as described in the **Supplementary Methods**. Briefly, we stimulated a coccygeal dorsal root (Co1) with a single pulse (0.1 ms, 0.02 mA, 3×T), recorded the reflex response on the S4 and Co1 sacrocaudal ventral roots, and computed the mean SLR (over 10–40 ms after stimulation), LLR (500–4,000 ms after stimulation) and background activity (over 300 ms before stimulation). For intracellular recordings, sharp intracellular electrodes were advanced into motoneurons, and the Ca^{2+} PIC was measured under voltage-clamp. The Ca^{2+} PIC was quantified as the downward current deflection (**Fig. 3b**, thick black line, at arrow) recorded during a slow upward voltage ramp (**Fig. 3b**, top, gray), relative to the leak current (thin line), in tetrodotoxin. Characteristically, this Ca^{2+} PIC was activated at low voltages (−56.7 ± 6.0 mV), deactivated at even lower voltages (on downward ramp) and mediated by L-type calcium channels (nimodipine-sensitive), as previously reported[12,15].

Locomotor assessment after spinal cord injury in rats. Locomotion was evaluated 3 weeks after the staggered hemisection using the BBB score[42], as detailed in the **Supplementary Methods**.

Drugs and solutions. The drugs used were 5-HT, fenfluramine, SB242084, strychnine, para-chlorophenylalanine-methyl-ester (all Sigma-Aldrich), α-methyl-5-HT, citalopram, cyproheptadine, methysergide, MK212, nimodipine, SB206553 (all Tocris) and tetrodotoxin citrate (Alomone). *In vitro*, the artificial cerebrospinal fluid consisted of (in mM) 122 NaCl, 24 $NaHCO_3$, 2.5 $CaCl_2$, 3 KCl, 1 $MgCl_2$ and 12 D-glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4) and maintained at 22–24 °C. Drugs were dissolved in the artificial cerebrospinal fluid. *In vivo*, drugs were administered via transcutaneous i.t. injection[60], intraperitoneal injection or oral gavage, and peak effects were reported (at 5–20 min after i.t. and 60 min after oral gavage). SB206553 was used at a dose that produced maximal effects (on spasms) both *in vivo* and *in vitro* (determined by titration), and SB242084 was used at the same dose, because SB206553 and SB242084 have similar binding affinity at 5-HT_{2C} receptors[49].

mRNA measurements. We extracted RNA from the whole spinal cord below the S2 injury level and from this synthesized and amplified cDNA (with RT-PCR) to quantify the mRNA. We quantified RNA editing and 5-HT_{2C} isoforms by sequencing the DNA of bacterial colonies grown from single bacteria cells transfected with DNA fragments synthesized and amplified from spinal cord cDNA (using 5-HT_{2C} receptor–related PCR primers; each colony adopts a single 5-HT_{2C} receptor isoform). We computed editing efficiency at each of five sites (A–E in **Fig. 4a**) as the proportion of colonies with editing at that site in their sequence. We computed the proportion of each 5-HT_{2C} receptor isoform in the spinal cord, from the number colonies with that isoform, relative to the total number of colonies. See further details in the **Supplementary Methods**.

Histology. Immunofluorescence labeling for 5-HT and 5-HT_{2C} receptors was performed as described in the **Supplementary Methods**.

Statistical analyses. Statistical comparisons were performed by a paired *t* test after verifying normality. Data are reported as means ± s.e.m.

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

60. Mestre, C., Pelissier, T., Fialip, J., Wilcox, G. & Eschalier, A. A method to perform direct transcutaneous intrathecal injection in rats. *J. Pharmacol. Toxicol. Methods* **32**, 197–200 (1994).