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Kv1.2 Channels Promote Nonlinear Spiking Motoneurons for Powering Up Locomotion

Graphical Abstract

Highlights
- Neonatal and adult bistable motoneurons display delayed spike-frequency acceleration
- Delayed spike-frequency acceleration reflects slow inactivation of Kv1.2 channels
- Kv1.2 channels are highly expressed in axon initial segments of motoneurons
- Slow inactivation of Kv1.2 channels amplifies motor outputs upon locomotion onset

Authors
Rémi Bos, Ronald M. Harris-Warrick, Cécile Brocard, ..., Daniel Zytnicki, Sergiy M. Korogod, Frédéric Brocard

Correspondence
frederic.brocard@univ-amu.fr

In Brief
Bos et al. demonstrate that slow inactivation of Kv1.2 channels is critical in shaping nonlinear firing properties in mammalian spinal cord. It provides a potent gain control mechanism in spinal motoneurons and has a behavioral role in enhancing locomotor drive during the transition from immobility to steady-state locomotion.
Kv1.2 Channels Promote Nonlinear Spiking Motoneurons for Powering Up Locomotion

Rémi Bos,1 Ronald M. Harris-Warrick,2 Cécile Brocard,1 Liliia E. Demianenko,4 Marin Manuel,3 Daniel Zytnicki,3 Sergiy M. Korogod,1 and Frédéric Brocard1,5,*

1Institut de Neurosciences de la Timone (UMR7289), Aix-Marseille Université and Centre National de la Recherche Scientifique (CNRS), Marseille, France
2Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA
3Centre de Neurophysique, Physiologie et Pathologie, UMR 8119, CNRS/Université Paris Descartes, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France
4Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine
5Lead Contact
*Correspondence: frederic.brocard@univ-amu.fr
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SUMMARY

Spinal motoneurons are endowed with nonlinear spiking behaviors manifested by a spike acceleration whose functional significance remains uncertain. Here, we show in rodent lumbar motoneurons that these nonlinear spiking properties do not rely only on activation of dendritic nifedipine-sensitive L-type Ca2+ channels, as assumed for decades, but also on the slow inactivation of a nifedipine-sensitive K+ current mediated by Kv1.2 channels that are highly expressed in axon initial segments. Specifically, the pharmacological and computational inhibition of Kv1.2 channels occluded the spike acceleration of rhythmically active motoneurons and the correlated slow buildup of rhythmic motor output recorded at the onset of locomotor-like activity. This study demonstrates that slow inactivation of Kv1.2 channels provides a potent gain control mechanism in mammalian spinal motoneurons and has a behavioral role in enhancing locomotor drive during the transition from immobility to steady-state locomotion.

INTRODUCTION

Spinal motoneurons are nonlinear integrators of the locomotor network (Brownstone, 2006; Heckman et al., 2008). The most distinctive nonlinear firing property consists of a self-sustained firing evoked by a brief excitation (Hougsaard and Mintz, 1988; Hultborn et al., 2013). This all-or-none bistable behavior arises from a prolonged depolarization known as a “plateau potential,” which is mediated by persistent inward currents (Bouhadfane et al., 2013; Hougsaard et al., 1984; Kiehn and Harris-Warrick, 1992; Schwindt and Crill, 1980). From extensive in vitro recordings, the plateau potential appears to be preceded by a slow subthreshold membrane depolarization, then manifested by a spike-frequency acceleration before reaching a steady-state firing rate (Bennett et al., 2001; Con-way et al., 1988; Hougsaard and Kiehn, 1989; Leroy et al., 2014) referred to as the “preferred firing range” (Kiehn and Eken, 1997). Analogous firing-frequency acceleration, seen in motor units from in vivo recordings, provides evidence that the slow voltage transition to plateau is part of the physiological repertoire of motoneurons (Collins et al., 2002, 2014; Eken et al., 2008; Kiehn and Eken, 1997; Nickolls et al., 2004). The other striking manifestation of the slow voltage transition to the plateau potential is the cumulative depolarization of the membrane potential and slow increase in spiking frequency with repetitive excitations at short intervals (Bennett et al., 1998a, 1998b; Svirskis and Hougsaard, 1997), as occurs during locomotion (Brownstone et al., 1994). This apparent short-term memory is usually referred to as a “windup” phenomenon. In vivo correlates are found in the form of a progressive amplification of both motor unit discharges and force development during the onset of repetitive movements like locomotion (Bennett et al., 1998a; Collins et al., 2002; Gorassini et al., 1999, 2002; Hornby et al., 2003; Kiehn and Eken, 1997; Nickolls et al., 2004).

The slow voltage transition to the plateau is assumed to rely on progressive recruitment of L-type Ca2+ channels, because both spike-frequency acceleration and windup are blocked by the L-type channel blocker nifedipine in motoneurons (Hornby et al., 2002; Hougsaard and Kiehn, 1989; Hougsaard and Mintz, 1988, 1997). However, the involvement of L-type Ca2+ channels has significant limitations during early developmental stages because both spike-frequency acceleration and windup emerge as early as birth in motoneurons of neonatal rats (Bouhadfane et al., 2013) when L-type Ca2+ channels are not or only weakly expressed (Gao and Ziskind-Conhaim, 1998; Hsiao et al., 1998; Svirskis and Hougsaard, 1997). This offset suggests that channels other than L-type Ca2+ channels may contribute to the appearance of nonlinear properties during early developmental stages. The present study demonstrates in rats and mice that the slow voltage transition to plateaus is mainly mediated by the slow inactivation of a nifedipine-sensitive K+ current through Kv1.2 channels. Furthermore, we take a step toward identifying a behavioral role for Kv1.2 channels in the windup of rhythmic motor outputs upon the initiation of locomotion.
Figure 1. The Delayed Spike-Frequency Acceleration from Bistable Motoneurons Reflects Basic Features of a TTX-Insensitive Slow Membrane Depolarization

(A) Superimposition of representative voltage traces in response to subliminal (bottom), liminal (middle black), or supraliminal (middle red) depolarizing pulses. Instantaneous frequency plots on top of intracellular recordings.

(B) Time-course changes in duration (gray dots, left y axis) and amplitude (black dots, right y axis) of afterhyperpolarizations (AHPs) during tonic spiking of the motoneuron illustrated in (A). (Top) Superimposed AHPs.

(C) Duration (gray dots, left y axis) and amplitude (black dots, right y axis) of AHPs as function of the firing frequency. Continuous lines are the best-fit linear regression. ***p < 0.001, Spearman correlation test. r indicates the correlation index.

(D) Similar recordings as in (A) under apamin (100 nM).

(E) Superimposed voltage traces in response to subliminal (left and middle) or supraliminal (right) depolarizing pulses before (black) and during (red) TTX (1 μM). The dotted line illustrates the spiking threshold (Vth).

(F) Mean peak amplitude of slow membrane depolarizations (4.3 ± 0.4 mV for control versus 4.4 ± 0.5 mV during TTX, n = 11 cells). p > 0.05, Wilcoxon paired test.

(legend continued on next page)
RESULTS

Delayed Spike-Frequency Acceleration: A Hallmark of Bistability Both in Neonatal and Adult Motoneurons

Under in vitro recording conditions with temperature >30°C and 1.2 mM CaCl2 in the saline as found in vivo (Fowler and Kellogg, 1975; Jones and Keep, 1988), up to 80% of neonatal motoneurons recorded in whole-cell mode from 4th/5th lumbar spinal cord slices display bistability, manifested by self-sustained spiking after a brief excitation (Bouhadfane et al., 2013). In response to subthreshold current steps, bistable lumbar motoneurons (n = 28 cells) invariably developed a voltage-dependent slow membrane depolarization triggered at −69.2 ± 0.6 mV. Close to the rheobase (888 ± 75 pA), the slow depolarization increased monoexponentially with a time constant of 4.6 ± 0.5 s (Figure 1A, bottom trace), and its maximum amplitude was 4.8 ± 0.3 mV. Slightly above rheobase, the ramp depolarization culminated in a delayed onset of spike discharge characterized by a net spike-frequency acceleration (Figure 1A, top black trace). Incremental depolarizing pulses reduced the delay until conversion to tonic spiking (Figure 1A, top red trace). The delayed firing occurred within a range of 1.00–1.25 ± 0.2 times the rheobase. At intermediate current pulses, some motoneurons (6 of 28) fired a burst of spikes at current onset, separated from tonic spiking by a pause (Figure S1A). During sustained discharge induced by a mean current of 1,100 ± 90 pA, spike frequency increased from 15.3 ± 0.8 to 24.8 ± 0.3 Hz until a steady-state rate was achieved without further apparent adaptation (p < 0.001; Figure S1B). During the discharge, motoneurons’ action potentials exhibited a progressive decrease of both duration and amplitude of the slow afterhyperpolarization (p < 0.001; Figure 1B; Figures S1C and S1D). These decreases paralleled the time course of acceleration of the firing frequency (Figure 1C; Figures S1E and S1F). Importantly, non-bistable motoneurons did not display either a voltage-dependent slow membrane depolarization or delayed spike-frequency acceleration. To determine whether this delayed firing pattern was a transitory developmental phenomenon, we recorded the firing properties of lumbar motoneurons from adult mice in vivo. Similar to the neonatal in vitro recordings, the majority of motoneurons (22 out of 30) displayed a slow membrane depolarization (Figure S2A) and started to fire with a delay from pulse onset. Once firing had started, the instantaneous firing frequency increased over time (Figure S2B).

Delayed Spike-Frequency Acceleration Reflects a TTX-Insensitive Slow Membrane Depolarization

We investigated the cellular mechanisms underlying the spike-frequency acceleration in neonatal rat motoneurons. Although the tight relationship between the time course of the afterhyperpolarization and discharge acceleration supports a role for Ca2+-activated K+ channels, their blockade by apamin did not abolish either the delayed spike-frequency acceleration or the subthreshold slow membrane depolarization (Figure 1D; Figures S3A–S3C). The slow membrane depolarization is not dependent on a subthreshold-activated persistent Na+ inward current because it was not affected by tetrodotoxin (TTX; 1 μM), a blocker of voltage-gated Na+ channels (p > 0.05; Figures 1E and 1F). Notably, the firing rate increase recorded before the application of TTX could be superimposed on the TTX-resistant slow membrane depolarization (Figure 1G). We thus considered that the apparent spike-frequency acceleration reflects basic features of the slow membrane depolarization independent of voltage-gated Na+ channels.

Subsequent experiments were conducted under TTX in order to determine the ionic basis of the slow membrane depolarization. Nifedipine (20 μM), a blocker widely used to demonstrate the contribution of L-type Ca2+ channels, significantly decreased the slow membrane depolarization (p < 0.05; Figure 1H). However, no sign of persistent inward Ca2+ current was clearly observed in our recordings (Figure S3D). Furthermore, the slow depolarization did not differ when recorded in Ca2+-free solution or in the presence cadmium (100 μM), a broad-spectrum Ca2+ channel blocker (p > 0.05; Figures 1I–1K). Neither the blockade of T-type Ca2+ channels by mibefradil (10 μM) nor intracellular Ca2+ chelation (1,2-bis(o-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid [BAPTA], 10 mM) affected the slow depolarization (p > 0.05; Figures S3E and S3F). We thus assumed that nifedipine did not block the slow depolarization by inhibiting Ca2+ influx, but by some other mechanism. In sum, both Ca2+-activated K+ channels and voltage-gated Ca2+ channels do not appear as major contributors of the TTX-insensitive slow membrane depolarization.

The Slow Membrane Depolarization Is Mediated by Slow Inactivation of a TEA-Insensitive K+ Current

In normal artificial cerebrospinal fluid (aCSF), during the spike-frequency acceleration, the action potential broadened monexponentially (p < 0.001; Figures 2A and 2B; Figure S4A). At the same time, the rise slope of spikes decreased linearly (p < 0.01; Figure 2C; Figure S4B), while the rate of repolarization monexponentially slowed (p < 0.001; Figure 2D; Figure S4C) and was linearly related to the time course of increase of both the duration of spikes (Figure 2E; Figure S4D) and the spike-frequency acceleration (Figure 2F; Figure S4E). This result led us to suspect that the spike-frequency acceleration and the slow membrane depolarization may be caused by a very slow inactivation of a K+ current. Under TTX, the broad-spectrum...
voltage-gated K⁺ channel blocker tetraethylammonium (TEA) increased the amplitude of the slow membrane depolarization \((p < 0.01; \text{Figure 2G})\). Thus, TEA-sensitive K⁺ currents appear to counteract the slow membrane depolarization. However, after an initial decrease by \(34\% \pm 8\%\) caused by the depolarizing step, the cell input resistance slowly increased by \(87\% \pm 18\%\) in parallel with the emergence of the slow depolarization \((p < 0.05; \text{Figure 2H})\). A slow decrease in membrane conductance accompanying a slow depolarization may be consistent with the slow inactivation of TEA-insensitive K⁺ channels. This hypothesis is further supported by a decrease in amplitude of the slow membrane depolarization when the extracellular K⁺ concentration was raised to 9 mM, which reduced the driving force on potassium in this voltage range \((p < 0.05; \text{Figure 2I})\).

Consistent with this, the reversal potential for the current was close to the predicted equilibrium potential for K⁺ \((-87.7 \pm 4.3\text{ mV}; \text{Figure S5A})\). We refer to this current as slow inactivating potassium outward current \((I_{Ks})\).

### Biophysical Properties of \(I_{Ks}\) Responsible for the Slow Membrane Depolarization

Experiments were undertaken on seven motoneurons to examine the voltage dependence of the channel(s) underlying \(I_{Ks}\). From the current-voltage relationship fitted with a standard Boltzmann function (\(I_{Ks}\), black traces), its threshold for activation was positive to \(-70\text{ mV}\), and its amplitude increased steeply (slope factor \(k\): \(7.5 \pm 0.7\)) for larger voltage steps with a midpoint of activation \((V_{1/2})\) at \(-43\pm 2\text{ mV}\) and then plateaued above \(-20\text{ mV}\) (\(V_{1/2}\), gray traces). The voltage dependence of inactivation was studied with a series of depolarizing presteps followed by measurement of the remaining current at \(-40\text{ mV}\) (\(I_{Ks}\), gray traces); \(I_{Ks}\) was half-inactivated at \(-51.9 \pm 2.2\text{ mV}\) and fully inactivated when the membrane potential was maintained above \(-30\text{ mV}\) (\(V_{1/2}\)). Recovery from inactivation was very slow: complete recovery of the initial amplitude potassium current required separation of two successive long-lasting current pulses by at least 12 s (\(V_{1/2}\), Figure S3E and S3F).
Figure 3. Biophysical Properties of Voltage-Gated K⁺ Channel(s) Responsible for the Slow Membrane Depolarization

(A and B) Superimposed outward currents elicited by a depolarizing step before and after extracellular perfusion of a medium containing 9 mM [K⁺]₀ (A) or after intracellular dialysis of cesium chloride (1 mM, B). (Right panels) Mean of current amplitude of slow inactivating outward currents indicated by the vertical arrows (354.1 ± 16.3 pA in 3 mM [K⁺]₀ versus 223.6 ± 11.5 pA in 9 mM [K⁺]₀, n = 7 cells; 236 ± 13.8 pA for control versus 0.40 ± 0.08 pA for CsCl, n = 7 cells). *p < 0.05, Wilcoxon paired test.

(C) Superimposed outward currents elicited by voltage steps from holding potential of −80 mV (left) or by stepping to the membrane potential of −20 mV from a series of holding potentials (right).

(D) Activation (black dots) and steady-state inactivation (gray dots) curves of outward currents (n = 7 cells).

(E) Recovery of outward currents from inactivation. The initial depolarization was followed by a second similar depolarizing step of same amplitude having interpulse interval from 1 to 15 s.

(F) Mean values of the fractional recovery as function of interpulse intervals normalized to the amplitude of the outward current elicited by the first depolarizing step.

(G) Representative outward current in response to voltage oscillations (0.5 Hz). In means, outward currents declined by 269.3 ± 30.2 pA, n = 6 cells. *p < 0.05, Wilcoxon paired test.

(H) Left panel: superimposed outward currents in response to a depolarizing pulse as a function of bath temperature (n = 5 cells). Right panels: mean current amplitude of slow inactivating outward currents as a function of temperature. Continuous line is the best-fit linear regression. r indicates the correlation index. All recordings are performed in the presence of TTX (1 µM) and TEA (10 mM). Data are mean ± SEM. See also Figure S5.
of the pulse and the onset of firing shortened in motoneurons from adult mice in vivo when the interval between two successive pulses was below 10 s, reflecting the reduction in \( I_{\text{KS}} \) during the second step at these shorter intervals (Figures S2B and S2C). A cumulative inactivation was also found with repeated short depolarizations. Thus, \( I_{\text{KS}} \) declined by 24\% ± 5.7\% when motoneurons were submitted to sinusoidal current injections at a frequency as low as 0.5–1 Hz (p < 0.05; Figure 3G). Note that the magnitude of \( I_{\text{KS}} \) linearly increased with temperature (Figure 3H) and was similar in identified motoneurons innervating the triceps surae or the tibialis anterior muscles (Figure S5C). In sum, irrespective of their functional identity, most motoneurons displayed a slow-inactivating temperature-sensitive K\(^+\) current likely responsible for the slow membrane depolarization.

The Pharmacological Profile of \( I_{\text{KS}} \) Implicates Kv1.2 Channels

To provide insight into the \( \alpha \) subunits forming the K\(^+\) channel(s), we applied a battery of K\(^+\) channel blockers in the presence of TTX and TEA. As a first observation, increasing the concentration of TEA from 10 to 20 mM did not further change \( I_{\text{KS}} \), suggesting that 10 mM was enough to block almost all TEA-sensitive K\(^+\) channels (p > 0.05; Figure S5D). In addition to blocking L-type Ca\(^{2+}\) channels, nifedipine inhibits a variety of K\(^+\) channels, including Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv2.1, and Kv3.1 (Grissmmer et al., 1994; Li et al., 2015). Nifedipine (20 \( \mu \)M) caused a reduction of \( I_{\text{KS}} \) (p < 0.05; Figure 4A; Table S1) and an inward shift of the holding current by \(-145 ± 30 \, \text{pA} \) (p < 0.05, n = 7 cells; Figure 4A). Spinal motoneurons of rats express Kv2.1 channels (Muenich and Fyffe, 2004), but their inhibition by stromatoxin (Escoubas et al., 2002) (STx; 3 \( \mu \)M) did not affect \( I_{\text{KS}} \) (p > 0.05; Figure 4B; Table S1).

After the pharmacological exclusion of Kv2 subunits, we assessed the response to 4-AP for which Kv1 channels are sensitive (Coetzee et al., 1999). 4-Aminopyridine (4-AP; 0.2 mM) reduced \( I_{\text{KS}} \) by the same amount as nifedipine (p < 0.05; Figure 4C; Table S1). To further investigate which Kv1 subunits are involved, we tested dendrotoxin-I (DTx-I; 1 \( \mu \)M), which blocks Kv1 channels that contain at least one Kv1.1 or Kv1.2 subunit (Grissmmer et al., 1994). DTx-I reversibly abolished \( I_{\text{KS}} \) (p < 0.01; Figure 4D; Table S1). Immunostaining substantiates the presence of Kv1.1 (Figures 4E and 4G) and Kv1.2 (Figures 4H–4J) channels in almost all presumptive neonatal motoneurons (57 out of 62 cells for Kv1.1; 62 out of 66 for Kv1.2), specifically in the distal part of their Ankyrin G-positive axonal initial segments. Both subunits were abundantly co-expressed in the vast majority of presumptive motoneurons (45 out of 49 cells; Figures 4K–4M).

To provide further clues about the channel subunit composition, we used toxins specific for one Kv1 subunit. Application of dendrotoxin-K (DTx-K; 1 \( \mu \)M), a potent blocker for Kv1.1 (Robertson et al., 1996), had no effect (p > 0.05; Figure 4N; Table S1), while tityustoxin (TsTX; 1 \( \mu \)M), which inhibits Kv1.2 (Werkman et al., 1993), reversibly suppressed \( I_{\text{KS}} \) (p < 0.01; Figure 4O; Table S1) and the slow depolarization it underlies (Figures 5A and 5B). Taken together, these data strongly implicate inactivation of potassium channels containing Kv1.2 subunits in the generation of \( I_{\text{KS}} \).

Inactivation of Kv1.2-Mediated \( I_{\text{KS}} \) Promotes Near-Threshold Nonlinear Firing Properties in Motoneurons

We tested the functional role of Kv1.2 channels on firing properties of bistable motoneurons by measuring their degree of spike-frequency acceleration before and after bath application of TsTX. Experiments were performed in the presence of the glutamate receptor blocker kynurenate (1.5 mM) to dampen neural network activity when Kv1.2 channels were blocked. As a first observation, bath application of TsTX increased the holding current required to maintain the membrane potential at \(-80 \, \text{mV} \) (p < 0.05; Table S2), consistent with the block of a Kv1.2-mediated conductance that is active at rest. As mentioned above, TsTX prevented or strongly decreased the slow membrane depolarization associated with \( I_{\text{KS}} \) (Figures 5A and 5B), as well as the delay in onset of firing, which can be observed across a narrow range of low-voltage steps up to 1.08 ± 0.06 times the rheobase (Figure 5C). In addition, the motoneuron’s slow acceleration of firing during tonic spiking was abolished with TsTX (Figures 5D and 5E); the neurons’ initial spike frequency was in the range of the maximal frequency seen at the end of the firing acceleration in untreated neurons. We further tested the role of Kv1.2 channels in the integration of rhythmic inputs, by investigating the motoneurons’ responsiveness to sine wave current injection of constant amplitude with temporal dynamics compatible with locomotor frequencies (0.5–2 Hz). In the subthreshold range, close to the rheobase, the voltage response of untreated motoneurons to eight successive same-amplitude cycles increased by 4.6 ± 0.4 mV (p < 0.01; Figure 5F), which was similar to the peak amplitude of the slow membrane depolarization seen in response to a rectangular current injection. When current was increased to reach the firing threshold, the first spike seen was elicited in response to the third or fourth cycle in the series (Figure 5G), and increased in frequency with each subsequent cycle. During application of TsTX, the slow accumulating depolarization of subthreshold oscillatory membrane potentials was occluded by TsTX (p < 0.01; Figure 5F); the response to the first cycle was the same as for all other cycles. Likewise, the delay of firing in response to successive oscillatory cycles disappeared (Figure 5G); the neurons fired at maximal frequency during the first oscillation. Note that all results described above are reproduced by 1 \( \mu \)M DTX-I (Figure S6; Table S2).

Kv1.2-Mediated \( I_{\text{KS}} \) Boosts Fictive Locomotion

To investigate the theoretical effect of Kv1.2 channels on motoneuron firing behavior, we used a multi-compartment computational model of the motoneuron. In this model, transient and persistent Na\(^+\) currents, voltage-dependent delayed rectification and Ca\(^{2+}\)-dependent K\(^+\) currents, and N-type Ca\(^{2+}\) current were expressed during the perinatal period (Gao and Ziskind-Conhaim, 1998), it was not included in the model. The model was further supplemented by a slowly inactivating Kv1.2-like conductance derived from our voltage-clamp recordings (Figures 3 and S7A–S7C). Simulated motoneurons expressing such currents reproduced key features of the biological responses to stepwise and sinusoidal depolarizing currents, i.e., a delayed firing pattern to small current steps (Figure 6A) and windup (increasing depolarization and acceleration of spiking)
Figure 4. The Pharmacological Profile of the Outward Current Associated with Immunostaining Implicates Kv1.2 Channels

(A–D) Superimposed outward currents in response to a depolarizing pulse before (black trace) and during (red trace) nifedipine (20 μM) (A), stromatoxin (STx; 3 μM) (B), 4-aminopyridine (4-AP; 0.2 mM) (C), or dendrotoxin-I (DTx-I; 1 μM) (D). On right of all panels, mean amplitude of slow inactivating outward currents indicated by the vertical arrows (259 ± 25 pA for control versus 60 ± 12 pA during nifedipine, n = 6 cells; 232 ± 27 pA for control versus 216 ± 35 pA during STx, n = 12 cells; 217 ± 25 pA for control versus 68.6 ± 18.2 pA during 4-AP, n = 6 cells; 163.7 ± 11.7 pA for control versus 18.4 ± 6.4 pA during DTx-I versus 162.5 ± 26.1 pA in washout, n = 8 cells), p > 0.05; *p < 0.05; **p < 0.01, Wilcoxon paired test in (A)–(C), one-way ANOVA with repeated measures in (D).

(E–J) Immunostaining of Kv1.1 (E) (n = 66 cells) or Kv1.2 (H, n = 62 cells) along the axon initial segments of neonatal rat lumbar motoneurons (L4-L5) labeled by the ankyrin G antibody (F and I). Kv1.1 and ankyrin G are merged in (G), and Kv1.2 and ankyrin G are merged in (J).

(K–M) Double immunostaining of Kv1.1 (K) and Kv1.2 (L) along the axon initial segments of neonatal rat lumbar motoneurons (L4-L5, n = 49 cells). Both are merged in (M). Asterisks indicate the nucleus of motoneurons. Arrowheads show axon initial segments. Scale bars, 20 μm.

(N and O) Superimposed outward currents in response to a depolarizing pulse before (black trace) and during (red trace) dendrotoxin-K (DTx-K; 1 μM) (N) or during tityustoxin (TsTX; 1 μM) (O). (Right panels) Mean amplitude of slow inactivating outward currents indicated by the vertical arrows (350.9 ± 53.6 pA for control versus 358.6 ± 57.8 pA during DTx-K, n = 6 cells; 291.5 ± 46.2 pA for control versus 75.6 ± 32.3 pA during TsTX versus 210.7 ± 39.7 pA during washout, n = 8 cells), p > 0.05; *p < 0.05; **p < 0.01, Wilcoxon paired test in (N), one-way ANOVA with repeated measures in (O). All recordings are performed in the presence of TTX (1 μM) and TEA (10 mM). Data are mean ± SEM.
during repeated oscillations (Figure 6B). Both phenomena were abolished if Kv1.2 current was “off,” mimicking the effects of TsTx (Figures 6C and 6D). Note that the delay between current onset and firing onset was shortened when time constant of the Kv1.2 current inactivation was accelerated (Figures S7D–S7G). In sum, the model supplemented with Kv1.2 current captures key features of near-threshold nonlinear spiking properties of motoneurons, making it suitable to use as a tool to explore how Kv1.2 channels might shape motor outputs during rhythmic activity such as locomotion.

We simulated a population of 50 uncoupled motoneurons that received sinusoidal stimuli representing incoming locomotor drive from the central pattern generator. To provide a necessary heterogeneity in firing properties of motoneurons, the half-activation ($V_{1/2}$) of the Kv1.2 current was Gaussian-distributed using our experimental estimates of 42.6 ± 1.6 mV. In the population, both the sequential number of the effective cycle and the number of spikes generated in each cycle randomly varied due to intra-population variability of Kv1.2 properties (Figure 6E, lower panel). As a result, the integrated activity builds up cycle by cycle before reaching a steady-state level (Figure 6E, upper panel).

To confirm the predictive functional role of slow inactivation of Kv1.2 channels in progressively boosting locomotor outputs, we performed ex vivo experiments from whole-mount spinal cord preparations with fictive locomotion evoked by stimulation of sensory afferents from the cauda equina (Figure 7A). As previously observed (Brocard et al., 2013), when locomotor-like activity developed in response to repeated caudal stimuli, the rhythmic motor outputs recorded in L5 ventral roots showed typical windup, characterized by progressive increases in ventral root burst amplitude ($p < 0.01$; Figure 7B). Direct application of aCSF from a pipette located above the L5 ventral horn column on one side of the spinal cord did not disturb this windup of fictive locomotor outputs ($p < 0.05$; Figure 7C). By contrast, a similar
application of either nifedipine (20 μM) or tityustoxin (1 μM) significantly occluded the windup of L5 ventral root discharges ipsilateral to the application (p > 0.05; Figures 7D and 7E, red traces) without affecting the untreated contralateral side (p < 0.01; Figures 7D and 7E; black traces); the first burst of fictive locomotor output was near the maximal amplitude seen after windup in the unaffected side. Altogether, these results establish a functional role for inactivation of Kv1.2 channels in progressively amplifying the initial output of fictive locomotion.

DISCUSSION

The present study shows that dynamic changes in motoneuronal excitability can be attributed to slow inactivation of Kv1.2 channels under physiological conditions. We found that most lumbar motoneurons display a delayed spike-frequency acceleration, as previously observed in neonatal mice (Leroy et al., 2014; Pambo-Pambo et al., 2009) likely in the largest fast-type motoneurons (Durand et al., 2015; Leroy et al., 2014). Far from being exclusive to spinal motoneurons, this firing pattern has been described in brainstem motoneurons of neonatal rats (Russier et al., 2003) and adult guinea pigs (Nishimura et al., 1989). Here, we show that this delayed spiking in motoneurons is not transiently expressed, but persists in a similar proportion into adulthood, suggesting that it develops early and is maintained through life.

Regardless of species-specific features, many factors may account for their late discovery in adults. First, our stimulation protocol (long-lasting subthreshold current) that most clearly demonstrated the slow membrane depolarization is infrequently used in vivo. Second, most intracellular recordings from in vitro adult preparations are performed at room temperature, which limits the activation of Kv1.2 channels (Russell et al., 1994). Third, adult slice preparations may bias recordings to small motoneurons with high input resistances (Carp et al., 2008) because larger delayed firing motoneurons that have large dendritic trees (Leroy et al., 2014) may suffer more damage to dendrites during the slice procedure. Finally, other currents whose effects on the firing pattern are opposed to those of IKS, such as currents involved in the spike-frequency adaptation (Iglesias et al., 2011; Miles et al., 2005), could be differentially neuromodulated depending on the preparation and on the animal state.

The delayed spike-frequency acceleration in motoneurons is linked to a slow ramp depolarization. Some studies indicate that the spike-frequency acceleration results from imbalance between changing inward and outward currents (Nisenbaum et al., 1994; Nishimura et al., 1989). The present study suggests that, in neonatal motoneurons, the activation of persistent inward currents may be less important than the inactivation of a K+ current. Perinatal motoneurons broadly express three types of K+ current, including the Ca2+-dependent K+ current, the A-current, and a TEA-sensitive slow-inactivating K+ current (Gao and Ziskind-Conhaim, 1998; Takahashi, 1990). While the last one may contribute to the slow depolarization in motoneurons (Leroy et al., 2015), we have identified a fourth K+ current whose inactivation plays the major role in generating the slow depolarization, and thereby the nonlinear spiking acceleration. This K+ current, here referred to as IKS, resembles in some ways the IKS current originally described in hippocampal cells (Storm, 1988): it is sensitive to 4-AP but insensitive to TEA, activates at subthreshold potentials, inactivates very slowly, and recovers from inactivation with a long time constant.
Our data point to Kv1.2 channel subunits as major contributors to $I_{Ks}$ in neonates. First, $I_{Ks}$ was eliminated by a Kv1.2-specific channel blocker and shows other pharmacological similarities with channels that contain Kv1.2 subunits. Second, immunostaining revealed that Kv1.2 channels were expressed in the initial segment of motoneurons. Third, a computational model endowed with a Kv1.2-like conductance reproduced motoneurons’ nonlinear firing properties. Last, $I_{Ks}$ has substantial similarities with the biophysical profile of the Kv1.2-encoded current in heterologous expression systems (Grissmer et al., 1994; Werkman et al., 1993).

Given the reported dependence of the firing frequency acceleration on L-type Ca$^{2+}$ channels in adults (Hornby et al., 2002; Hounsgaard and Kiehn, 1989; Hounsgaard and Mintz, 1988; Hsiao et al., 1998; Svirskis and Hounsgaard, 1997), it is possible that $I_{Ks}$ is a transient characteristic at a time when L-type Ca$^{2+}$ channels are weakly expressed in neonatal rat motoneurons (Gao and Ziskind-Conhaim, 1998). Our data provide support to the view that adult motoneurons do express functional Kv1.2 channels. Although the pharmacological approach to assess their contribution is quite challenging in vivo, the delayed firing pattern mediated in neonates by Kv1.2 channels appears to be qualitatively similar to that recorded in adult motoneurons. Notably, the progressive reduction of the delayed onset of firing in response to repetitive stimuli fits well with the rate of slow recovery from inactivation of Kv1.2 channels. Consistent with our findings, adult lumbar motoneurons in mammals express both Kv1.2 channels in their initial segments (Duflocq et al., 2011; Rasband and Trimmer, 2001) and an unidentified
TEA-insensitive slow inactivating K⁺ current (Schwindt and Crill, 1981). In sum, in adult motoneurons, Kv1.2 and L-type Ca²⁺ channels may have a complementary role in nonlinear firing properties; the L-type Ca²⁺ channels highly expressed in dendrites (Jiang et al., 1999) may amplify synaptic inputs, while inactivation of Kv1.2 channels highly expressed in initial segments (Duflocq et al., 2011; Rasband and Trimmer, 2001) may boost motoneuronal output.

\( I_{\text{Ks}} \) mediated by Kv1.2 channels powerfully regulates the excitability of motoneurons in many ways. We found it to contribute to setting the resting membrane potential, to shunt early excitation manifested by an initial pause in firing, or a pause before initiation of firing, and then to promote a slow acceleration in firing rate because of its slow inactivation. This spike frequency acceleration preceding the initiation of plateau potentials is a marker of bistable motoneurons attributed to activation of a nifedipine-sensitive L-type Ca²⁺ current as well as persistent sodium and TRP-family calcium-activated nonsensitive \( (I_{\text{CaNN}}) \) currents (Bouhadfane et al., 2013; Hornby et al., 2002; Houngaard and Kiehn, 1989; Houngaard and Mintz, 1988). It is not widely known that nifedipine also blocks potassium channels including Kv1.2 channels (Grissmer et al., 1994). In light of this and our results, Kv1.2 channels appear to be a significant determinant in the voltage transition to the plateau potential. We can reasonably assume that the slow inactivation of Kv1.2 channels provides the initial depolarization that in turn initiates the recruitment of persistent inward currents sustaining plateau potentials. Under this assumption, the slow inactivation of Kv1.2 channels may be the primary mechanism by which the plateau threshold is lowered during tonic synaptic excitation (Bennett et al., 1998b).

Finally, another important feature of Kv1.2 channels is their slow recovery from inactivation, which provides to motoneurons a memory trace of their own activity. As a result, successive excitations in motoneurons become more efficient to reach the firing level (Leroy et al., 2015).

Genetic studies have supported the importance of Kv1.2 channels for motor function by linking their mutations to movement and gait disorders (Syrbe et al., 2015; Xie et al., 2010), but the cellular mechanism behind this was unclear. The present study provides insights into the operation of the locomotor network with a critical implication of Kv1.2 channels in adjusting the gain of motoneurons to behavioral needs. In motor tasks that involve repetitive movements such as locomotion, we show that the cumulative inactivation of Kv1.2 channels underlies windup of rhythmic motor outputs upon the initiation of fictive locomotion, as reported during the onset of step movements in cats (Jell et al., 1985) and humans (Gerasimenko et al., 2015). Kv1.2 channels may also contribute to the short-term potentiation of locomotor drives in motoneurons (Brownstone et al., 1994), somewhat reflected in the temporal facilitation of muscle activity produced by rhythmic muscle stretches in awake animals (Bennett et al., 1998a; Gorassini et al., 1999; Hornby et al., 2003) or by repetitive voluntary contractions or muscle vibrations in humans (Gorassini et al., 1998, 2002; Hornby et al., 2003; Romainguè et al., 1993; Suzuki et al., 1990). In motor tasks that instead involve a tonic recruitment of motoneurons, such as during posture, we show that the slow inactivation of Kv1.2 initiates a voltage transition to a delayed nonlinear spiking activity and facilitates prolonged higher-frequency firing rates. Likewise, motor units in humans are sometimes recruited with a quite robust delay (which may exceed 10 s) followed by a firing acceleration and a gradation of muscle force (Desmedt and Godaux, 1975; Kiehn and Eken, 1997). Thus, Kv1.2 channels may be useful in generating smooth control of the onset of muscle output by helping motoneurons to reach their “preferred firing range” (Kiehn and Eken, 1997). Finally, in behavioral contexts that involve a phasic recruitment of motoneurons such as during motor reflexes, the fast activation of Kv1.2 channels may serve as a low-pass filter, allowing motoneurons to respond preferentially to synchronous large-amplitude inputs while filtering out the small ones. Such circumstance may occur when large inputs from spindle primary afferents depolarize motoneurons to trigger a stretch reflex.

To conclude, in addition to mediating the nonlinear spiking properties that are markers of bistable motoneurons, we suggest that Kv1.2 channels play a fundamental role in the dynamics of locomotor circuits by switching motoneurons between gating and amplifying modes.

**EXPERIMENTAL PROCEDURES**

Further details and an outline of resources used in this work can be found in the Supplemental Experimental Procedures.

**Animals**

Neonatal (3–11 days old) Wistar rats and adult mice (18 B6SJL, 12 C57BL/6, 45–180 days old) were housed under a 12-hr light/dark cycle in a temperature- and humidity-controlled area with ad libitum access to water and food. All animal care and use conformed to the French regulations (Décret 2010–118) and were approved by the INT Marseille ethics committee CEEA 71 (authorization Nb A9 01 13) for the rat experiments and by Paris Descartes University ethics committee (authorizations CEEA34.MM.064.12 and 01256.02) for the mice experiments.

**In Vitro Models**

Slice preparation and whole spinal cord preparation were used for the whole-cell recordings and fictive locomotion experiments, respectively. Preparation procedures are detailed in the Supplemental Experimental Procedures.

**In Vivo Model**

Anesthetized in vivo preparations were used for lumbar motoneuron recordings in adult mice. Preparation procedures are detailed in the Supplemental Experimental Procedures.

**Intracellular Recordings**

From in vitro experiments, whole-cell patch-clamp recordings were made from L4–L5 ventrolateral lumbar motoneurons. From in vivo experiments, intracellular recordings from lumbar motoneurons were performed with sharp electrodes. Procedures of intracellular recordings are detailed in the Supplemental Experimental Procedures.

**Extracellular Recordings**

Motor outputs were recorded using extracellular stainless steel electrodes placed in contact with right and left lumbar L5 ventral roots in response to spinal caudal equina stimulation via a suction electrode. See the Supplemental Experimental Procedures for more details.

**Simulations**

Simulations were performed in the NEURON simulation environment on a multi-compartmental motoneuron model. See the Supplemental Experimental Procedures for more details.
Immunohistochemistry
Transverse spinal cord sections at the lumbar L4-L5 level were processed for immunohistochemistry using antibodies against Ankyrin G, Kv1.1, Kv1.2 channel isoforms. Tissue processing and staining are detailed in the Supplemental Experimental Procedures.

Statistical Analysis
The sample size was estimated considering the variation and mean of the samples. No statistical method was used to predetermine sample size. We used a nonparametric Mann-Whitney test or a Wilcoxon matched pairs test when two groups were compared, and a one-way ANOVA with or without repeated measures for multiple-group comparisons (GraphPad Prism 5 software). For all statistical analyses, the data met the assumptions of the test, and the variance between the statistically compared groups was similar. p values <0.05 were considered significant. As mentioned in the figure legends, all data are presented as mean ± SEM.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.02.093.

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AUTHOR CONTRIBUTIONS
R.B. designed, performed, and analyzed in vitro experiments. R.M.H.-W. designed and performed some of the in vitro experiments. C.B. designed and performed the immunohistochemistry. L.D. and S.K. co-designed the cell model and performed the analysis. M.M. and D.Z. co-designed and performed the immunohistochemistry. L.D. and S.K. co-designed the cell model and performed the analysis. M.M. and D.Z. co-designed and performed the analysis. M.M. and D.Z. co-designed and performed the analysis. M.M. and D.Z. co-designed and performed the analysis. M.M. and D.Z. co-designed and performed SEM.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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Supplemental Information

Kv1.2 Channels Promote Nonlinear Spiking Motoneurons for Powering Up Locomotion

Rémi Bos, Ronald M. Harris-Warrick, Cécile Brocard, Liliia E. Demianenko, Marin Manuel, Daniel Zytnicki, Sergiy M. Korogod, and Frédéric Brocard
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Frédéric Brocard (frederic.brocard@univ-amu.fr).

EXPERIMENTAL MODELS

Animal models.

Experiments were performed on neonatal (3- to 11-d-old) Wistar rats of either sex. Experiments were also performed on 30 adult mice of either sex (18 B6SJL and 12 C57BL/6) aged between 45 and 180 days old. We housed rodents in a temperature-controlled animal care facility with a 12 h light/dark cycle. We undertook all efforts to minimize animal suffering and the number of animals used. All animal care and use conformed to the French regulations (Décret 2010-118) and were approved by the local ethics committee CEEA 71-Comité d'éthique en neurosciences - INT Marseille (authorization Nb A9 01 13) for the rats, and by Paris Descartes University ethics committee (authorizations CEEA34.MM.064.12 and 01256.02) for the mice experiments.

In vitro models.

Details of the in vitro preparations have been previously described (Bouhadfane et al., 2015; Vinay et al., 1999) and are only summarized here. For the whole spinal cord preparation, the spinal cord was transected at T10, isolated and transferred to the recording chamber perfused with an oxygenated normal Ringers solution composed of the following (in mM): 120 NaCl, 4 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 1.2 CaCl₂, 25 NaHCO₃, 20 D-glucose, pH 7.4 (24-26°C). For the slice preparation, the lumbar spinal cord was isolated in ice-cold (+4°C) artificial CSF (ACSF) solution composed of the following (in mM): 232 sucrose, 3 KCl, 1.25 KH₂PO₄, 4 MgSO₄, 0.2 CaCl₂, 26 NaHCO₃, 25 D-glucose, pH 7.4. The lumbar spinal cord was then introduced into a 1% agar solution, quickly cooled, mounted in a vibrating microtome (Leica, VT1000S) and sliced (300-350 µm) through the L4–5 lumbar segments.
Slices were immediately transferred into the holding chamber filled with ACSF solution composed of the following (in mM): 120 NaCl, 3 KCl, 1.25 NaH2PO4, 1.3 MgSO4, 1.2 CaCl2, 25 NaHCO3, 20 D-glucose, pH 7.4, 30-32°C. Following a 30 min-1 h resting period, individual slices were transferred to a recording chamber that was continuously perfused (4 ml/min) with ACSF heated to 32-34°C composed of the following (in mM): 145 NaCl, 3 KCl, 1.3 MgCl2, 1.2 CaCl2, HEPES 10, 20 D-glucose, pH 7.4, 32-34°C). The temperature regulation was provided by the CL-100 bipolar temperature controller (Warner Instruments).

In vivo model.

Details of the in vivo preparation have been published previously (Delestree et al., 2014; Manuel et al., 2009; Manuel and Heckman, 2011). Briefly, atropine (0.2 mg/kg) mixed in perfusion solution (4% glucose, 1% NaHCO3, 14% Gelatin, pH 7) was given subcutaneously at the onset of the experiment to prevent salivation. After ten minutes, anesthesia was administered intraperitoneally with sodium pentobarbital (70 mg/kg). After confirming the absence of noxious reflexes, a tracheotomy was performed and the mouse was artificially ventilated with 100% oxygen with a SAR-830/AP ventilator (CWE, Ardmore, PA). The end tidal PCO2 was continuously monitored and maintained around 4% (MicroCapstar; CWE). Body temperature was maintained at 37°C using a heating blanket supplemented by an infrared heating lamp and monitored using a rectal temperature probe. The heart rate was monitored (CT-1000; CWE) and maintained between 400 and 500 bpm. A catheter was placed in the jugular vein for supplemental anesthesia (6 mg/kg, every 10–15 minutes) mixed in perfusion solution. Depth of anesthesia was assessed by a lack of noxious reflexes, a stable heart rate, and a stable end-tidal PCO2, showing no signs of resistance against the artificial ventilation. The vertebral column was immobilized with two pairs of horizontal bars (Cunningham Spinal Adaptor; Stoelting, Dublin, Ireland) applied on the Th13 and L2 vertebral bodies, and the L2–L4 spinal segments were exposed by a laminectomy beginning at the T13–L1 level. A custom made chamber was fit around the exposed spinal segments and silicon sealant (WPI, Sarasota, FL) was applied to create a recording chamber. This
chamber was filled with mineral oil to prevent dehydration of the spinal cord. Hindlimb nerves were dissected and mounted on bipolar electrodes for stimulation and identification of motoneurons through antidromic activation.

METHOD DETAILS

**Method to label motoneurons.**

The procedure to label motoneurons before slice preparation was previously described in detail (Sadlaoud et al., 2010). Briefly, in cryoanesthetized rat pups, 3-5 µl of a solution of fluorescein conjugated cholera toxin B subunit (Sigma-Aldrich, 0.5 mg/ml) was injected bilaterally into the triceps surae muscles or tibialis anterior to retrogradely label gastrocnemius/soleus and tibialis motoneuron pools, respectively. Animals were returned to their cages and a post-injection survival time of at least 12 h enabled retrograde transport to the cell bodies for visualization in slices.

**In vitro recordings and stimulation**

Electrophysiological data were acquired and digitized at 10 kHz through a Digidata 1440a interface using Clampex 10 software (Molecular Devices). *From the whole spinal cord preparation*, motor outputs were recorded using extracellular stainless steel electrodes placed in contact with right and left L5 ventral roots and insulated with Vaseline. The ventral root recordings were amplified (×2,000) and filtered (70 Hz to 3 kHz). The locomotor-like activity was induced by an electrical stimulation (dashed lines in Figure 7) delivered to the caudal equina of the spinal cord via a suction electrode. To determine the stimulus threshold (T), single pulses were delivered at increasing intensities until a polysynaptic reflex response was elicited. A pulse-train threshold (1-4 Hz for 20 s, 200-850 µA range, 0.2 ms pulse duration) was empirically determined to elicit locomotor-like activity episodes, and the spinal cord was allowed to rest for at least 5 min between two consecutive evoked episodes. *From the slice preparation*, motoneurons were visualized with epifluorescence and infrared differential interference contrast microscopy using a Nikon Eclipse E600FN upright
microscope coupled with a water-immersion objective (Nikon Fluor 40X/0.8W). Whole-cell patch-clamp recordings were mostly made from unidentified motoneurons defined as large cells located in the lateral ventral horn but also from motoneurons innervating the triceps surae or the tibialis anterior muscles back labeled with a fluorescein-conjugated cholera toxin (as described previously). The image was enhanced with a Hitachi KP-200/201 infrared-sensitive CCD camera and displayed on a video monitor. Whole-cell patch-clamp recordings in current- or voltage-clamp mode were performed with a Multiclamp 700B amplifier (Molecular Devices). Patch electrodes (2–4 MΩ) were pulled from borosilicate glass capillaries (1.5 mm OD, 1.12 mm ID; World Precision Instruments) on a Sutter P-97 puller (Sutter Instruments) and filled with intracellular solution containing the following (in mM): 140 K+-gluconate, 5 NaCl, 2 MgCl₂, 10 HEPES, 0.5 EGTA, 2 ATP, 0.4 GTP, pH 7.3 (280-290 mOsm). Pipette and neuronal capacitive currents were canceled, and after breakthrough, the series resistance was compensated and monitored. Access resistance was monitored periodically throughout the experiments. Neurons recorded in current-clamp mode with Tityustoxin or Dendrodotoxin-I were isolated from glutamatergic excitatory inputs with kynurenic acid (1.5 mM). Different voltage protocols were used to characterize the biophysical properties of the outward current. For the voltage dependence of activation, the membrane potential was initially held in voltage clamp at -80 mV and then stepped for 7.5 s to positive potentials with 30-45 s intervals between each pulse (Figure 3A). The interval between each paired pulse was at least of 30 s to avoid accumulation of the inactivation. The voltage dependence of steady-state inactivation was also obtained by a series of 30 second prepulses ranging from -80 to -20 mV, followed by a fixed test pulse to -20 mV (Figure 3B). A voltage protocol was also conducted to explore the rate of the current’s recovery from inactivation. For this, currents were first evoked by a 7.5 s long pulse to -40 mV. Subsequently, motoneurons were hyperpolarized to -80 mV for 1 s to 15 s, and finally stepped back to -40 mV to assess the extent of recovery (Figure 3C). All experiments were designed to gather data within stable period (i.e. at least 5 min after establishing whole-cell access) and cells with obvious run-down of the current were discarded. Measurement of the
reversal potential of K⁺ was performed in presence of a blocker of HCN channels, ZD7288 (20 μM), to minimize contamination from the hyperpolarization-activated K⁺-sensitive Ih current.

**In vivo recordings.**

Intracellular recordings were performed using glass microelectrodes (tip diameter 1.0–1.5 μm) filled with KCl 3M (resistance 8–15 MΩ). Intracellular recordings were obtained with an Axoclamp 2B amplifier and Spike2 software (CED, Cambridge, England). All motoneurons included were identified by antidromic stimulation of their axons. All motoneurons retained for analysis had a resting membrane potential more hyperpolarized than −50 mV and an overshooting action potential. All recordings were obtained in discontinuous current clamp mode (8–10 kHz) and sampled at 20 kHz.

**Simulations**

Simulations were performed in the NEURON simulation environment on a multi-compartmental motoneuron model, composed of the reconstructed dendritic arborization (Amendola and Durand, 2008; Filipchuk and Durand, 2012), cylindrical soma (length L= 25 μm, diameter d=35 μm), axon hillock (L=10 μm, tapering d=5.5 to 1.1 μm), initial segment (L=15 μm, d=1.1 μm) and stem axon (d=1.1 μm) of 20 myelinated 200 μm long segments with 1 μm nodes of Ranvier. The dendritic membrane was considered passive (the leak conductivity $G_{\text{Leak}} = 0.04$ mS/cm², if not indicated otherwise) and the reversal potential was fixed at $E_{\text{Leak}} = -70$ mV. The soma contained ion channels that conducted currents of seven types: fast inactivating tetrodotoxin-sensitive sodium ($I_{\text{Na-TTX}}$); non-inactivating persistent sodium ($I_{\text{NaP}}$); high-voltage-activated N-type calcium ($I_{\text{CaN}}$); fast delayed rectification potassium ($I_{\text{KDR}}$); rapidly activating slowly inactivating Kv1.2-type potassium ($I_{\text{Kv1,2}}$); small conductance calcium-dependent potassium ($I_{\text{SK}}$); and leak current ($I_{\text{Leak}}$). $I_{\text{Na-TTX}}$, $I_{\text{KDR}}$, $I_{\text{Kv1,2}}$, and $I_{\text{Leak}}$ currents were included in the axon hillock and initial segment. The same currents except $I_{\text{Kv1,2}}$ were included in the nodes of Ranvier. The currents were described by the
Hodgkin-Huxley-type equations (see Supplemental information). Our model of Kv1.2-type potassium channels was based on the experimental data collected in the present study. The cell was stimulated by depolarizing currents applied at the soma. Responses were recorded from the soma and distal end axon. The recorded sequences of action potentials were quantitatively characterized and graphically represented by momentary frequency and raster plots. A special set of computation experiments was performed on 50 uncoupled simulated motoneurons of the same geometry. Intra-population variability was simulated by randomization of the half-activation potential of Kv1.2 current $V_{1/2}$, which had a Gaussian distribution of $42.6 \pm 1.6$ mV that corresponded to the experimental estimates. All cells received the same sinusoidal current stimulation. The activity of the population was represented by a sequence of raster plots of individual cells spiking where each horizontal line represented a neuron and each dot represented a spike. From these data the integrated population activity was derived and represented by the histogram of average number of spikes per second per neuron (bin width 10 ms).

**Immunohistochemistry.**

Spinal cords of 7 day-old rats were dissected out and fixed for 1 h in 0.25% paraformaldehyde (PFA), then rinsed in phosphate buffered saline (PBS) and cryoprotected overnight in 20% sucrose at 4°C. Spinal cords were frozen in OCT medium (Tissue Tec) and 25 µm cryosections were collected from the L4-L5 segments. Slides were washed 3×5 min in Tris-buffered saline (TBS). Slides were incubated for 1 h in a blocking solution (10% horse serum in TBS) with 0.4% triton X-100 and overnight at 4 °C in a humidified chamber with the primary antibodies, diluted in the blocking solution with 0.2% triton X-100. Slides were then washed 3×5 min in TBS and incubated for 1 h with the secondary antibodies, diluted in the blocking solution with 0.2% triton X-100. After washing 3×5 min in TBS, slides were mounted with a gelatinous aqueous medium. Images were acquired using a confocal microscope (LSM510, Zeiss) equipped with a 40x oil objective and processed with the ImageJ software. Each figure corresponds to a projection image from a stack of optical sections.
**Antibodies.**

The following antibodies were used: anti- Kv1.1 mouse monoclonal (Agent Cat# 75-007; RRID:AB_10673165) diluted 1/200; anti-Kv1.2 mouse monoclonal (Agent Cat# 75-008; RRID:AB_2296313) diluted 1/200; anti-Ankyrin G rabbit polyclonal (Agent CAT# SC-28561; RRID:AB_633909H-125) diluted 1/200. Mouse monoclonal antibodies were detected by the application of the isotype-specific secondary antibodies, AlexaFluor-488 goat anti-mouse IgG2b (Agent CAT# A-21141; RRID:AB_141626), AlexaFluor-555 goat anti-mouse IgG1 (Agent CAT# A-21127; RRID:AB_141596); Rabbit polyclonal antibody was detected by AlexaFluor-546 F(ab')2 goat anti-rabbit IgG (Agent Cat# A-11071; RRID:AB_2534115).

**Drug list.**

Normal aCSF was used in most cases for electrophysiological recordings. High-K⁺ aCSF was prepared by adding 1M KCl solution. Ca²⁺-free solution was made by removing Ca²⁺ chloride from the recording solution and replacing it with an equimolar concentration of magnesium chloride. The following pharmacological agents from Sigma-Aldrich were used: apamin (100 nM), BAPTA (10 mM), Mibefradil (10 µM), Cesium Chloride (120 mM), Cadmium Chloride (100 µM) Tetraethylammonium chloride (TEA; 10-20 mM), Kynurenic acid (1.5 mM), 4-Aminopyridine (0.2 mM), ZD 7288 (20 µM). Tetrodotoxin citrate (TTX; 1 µM) were purchased from Tocris Bioscience Biotechne; Dendrotoxin-I and Tityustoxin K (1 µM) from Alomone labs; Dendrotoxin-K (1 µM) from EnzoLife Science and Stromatoxin-1 (0.6-3 µM) from Smartox biotechnology. Nifedipine (20 µM, Sigma-Aldrich) was dissolved in dimethylsulphoxide (DMSO) and added to the ACSF (final concentration of DMSO: 0.05–0.1%). In some recordings 10 mM BAPTA was added in the pipette solution to chelate intracellular Ca²⁺; in others a cesium-based intracellular solution was used to block voltage-dependent K⁺ channels (in mM): 110 CsCl, 30 K-gluconate, 5 NaCl, 2 MgCl2, 10 HEPES, 0.5 EGTA, 2 ATP, 0.4 GTP, pH 7.3 (280–290 mOsm). In some experiments using the *in vitro*
whole spinal cord preparation, a pipette with a wide drip tip containing nifedipine (20-40 µM) or Tityustoxin K (1 µM) was placed within the ventral horn. Leakage of the solution from the pipette was monitored by including neutral red in the intrapipette solution.

**Data analysis.**

Electrophysiological data were analyzed off-line with Clampfit 10 software (Molecular Devices). Alternating activity between right/left L5 recordings was taken to be indicative of fictive locomotion. To characterize locomotor burst parameters, raw extracellular recordings from ventral roots were rectified and resampled at 50 Hz. The amplitude of ventral root bursts was measured by a threshold function which determines the peak of bursts of activity (Brocard et al., 2013). Several basic criteria were set to ensure optimum quality of intracellular recordings. Only cells exhibiting a stable resting, holding membrane potential, access resistance (less than 20% variation) and an action potential amplitude larger than 45 mV were considered. All reported membrane potentials were corrected for liquid junction potentials. Properties of the slow depolarization were measured from at least two 7.5 s-long depolarizing current pulses close to the rheobase. The maximum amplitude of the slow depolarization corresponds to the difference between the potential at the onset of the response and the potential measured just below the spiking threshold. Firing properties were investigated with 7.5 s-long depolarizing current pulses of varying amplitudes. The instantaneous discharge frequency was determined as the inverse of interspike interval and plotted as a function of time. Passive membrane properties of cells were measured by determining from the holding potential the largest voltage deflections induced by small currents pulses that avoided activation of voltage-sensitive currents. The rheobase was defined as the minimum step current intensity required to induce an action potential from the membrane potential held at -80 mV. Peak spike amplitude was measured from the threshold potential, and spike duration was measured at half-amplitude. The peak amplitude and duration (to half-amplitude) of slow afterhyperpolarizations were measured from the action potential threshold. In some experiments, for a direct comparison of firing properties or slow
membrane depolarizations before and during the application of the drug, current pulses were adjusted to reach the same level of depolarization at the onset of the pulse as that for control. If necessary, using bias currents, the pre-pulse membrane potential was maintained at the holding potential fixed in the control condition. For measurements of the slow inactivating outward current, the peak of the whole-cell current was measured 200 ms after the onset of the pulse to avoid the contribution of the fast inactivating K⁺ current Iₐ. The residual current at the end of the depolarizing pulse was taken as a measure of the noninactivating component and was subtracted from the peak outward current, to yield an estimate of the inactivating component. Voltage dependence and recovery of the inactivating current were analyzed from data normalized to the maximal current. The I/V curves were fitted with a Boltzmann function, and the curve for recovery from inactivation with a mono-exponential function. For kinetics of the current, time constants were determined by fitting an exponential function to the rising or decay phase of the voltage trace.

DATA AVAILABILITY.

All data generated and/or analyzed during this study are included in this published article and its supplemental information files.

RESOURCE TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| anti-Kv1.1          | UC Davis/NIH NeuroMab Facility | Cat# 75-007 RRID:AB_10673165 |
| anti-Kv1.2          | UC Davis/NIH NeuroMab Facility | Cat# 75-008 RRID:AB_2296313 |
| anti-Ankyrin G      | Santa Cruz Biotechnology | CAT# SC-28561 RRID:AB_633909 |
| AlexaFluor-488 goat anti-mouse IgG2b | Thermo Fisher Scientific | CAT# A-21141 RRID:AB_141626 |
| AlexaFluor-555 goat anti-mouse IgG3 | Thermo Fisher Scientific | CAT# A-21127 RRID:AB_141596 |
| AlexaFluor-546 F(ab')2 goat anti-rabbit IgG | Thermo Fisher Scientific | Cat# A-11071 RRID:AB_2534115 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| NaCl                | Sigma-Aldrich | CAT# 71376 |
| KCl                 | Sigma-Aldrich | CAT# P3911 |
| NaH₂PO₄             | Sigma-Aldrich | CAT# 50751 |
| MgSO₄               | Sigma-Aldrich | CAT# 1880 |
| CaCl₂               | Sigma-Aldrich | CAT# 21115 |
| Chemical          | Supplier       | CAT#      |
|-------------------|----------------|-----------|
| NaHCO₃            | Sigma-Aldrich  | S6014     |
| D-glucose         | Sigma-Aldrich  | G8270     |
| K⁺-gluconate      | Sigma-Aldrich  | P1847     |
| MgCl₂             | Sigma-Aldrich  | M8266     |
| HEPES             | Sigma-Aldrich  | H3375     |
| EGTA              | Sigma-Aldrich  | E3889     |
| ATP               | Sigma-Aldrich  | A9062     |
| GTP               | Sigma-Aldrich  | G9002     |
| Sucrose           | Sigma-Aldrich  | S9375     |
| Apamin            | Sigma-Aldrich  | A9459     |
| BAPTA             | Sigma-Aldrich  | A4926     |
| Mibefradil        | Sigma-Aldrich  | M5441     |
| Cesium Chloride   | Sigma-Aldrich  | 203025    |
| Cadmium Chloride  | Sigma-Aldrich  | 202908    |
| Tetraethylammonium chloride | Sigma-Aldrich | 86616     |
| Kynurenic acid    | Sigma-Aldrich  | K3375     |
| 4-Aminopyridine   | Sigma-Aldrich  | 275875    |
| ZD 7288           | Sigma-Aldrich  | 23777     |
| Nifedipine        | Sigma-Aldrich  | N7634     |
| Dimethylsulphoxide| Sigma-Aldrich  | D8418     |
| Tetrodotoxin citrate | Tocris Bioscience Biotchnology | CAT# 1069 |
| Dendrotoxin-I     | Alomone labs   | D-390     |
| Tityustoxin K     | Alomone labs   | STT-360   |
| Dendrotoxin-K     | EnzoLife Science | BML-KC164-0070 |
| Stromatoxin-1     | Smartox biotechnology | CAT# SCT01-00100 |
| Robinul-V (Atropine) | Vetoquinol       | N/A       |
| Sodium pentobarbital | Ceva             | N/A       |
| Paraformaldehyde  | EMS             | 15714-S   |
| Phosphate Buffered Saline | Argene Biométeux | 33-011  |
| Tissue-Tek OCT compound | VWR             | 25608-930 |
| Tris-buffered saline | Bio-world       | 105300272 |
| Triton X-100      | Sigma-Aldrich  | T9284     |
| Cholera toxin B subunit | Sigma-Aldrich | C1655     |

**Experimental Models: Organisms/Strains**

| Organism          | Supplier          | RRID     |
|-------------------|-------------------|----------|
| Wistar rats        | Charles River Laboratories | RGD_2308816 |
| B6SJL mice         | Janvier labs      | N/A      |
| C57BL/6 mice       | Charles River Laboratories | IMSR_CRL:643 |

**Software and Algorithms**

| Software          | Version          | RRID     |
|-------------------|------------------|----------|
| Clampex 10        | v10.3            |          |
| Clampfit 10       | v10.3            |          |
| Spike2            | CED, Cambridge, England | v 5.21  |
| ImageJ            | https://imagej.nih.gov/ij/ | v1.50i   |
| Graphpad Prism    | Prism            | SCR_002798 |
| NEURON            | https://www.neuron.yale.edu/neuron/ | v7.5    |

**Other**

| Equipment                     | Manufacturer     | Model    |
|-------------------------------|------------------|----------|
| Vibrating microtome           | Leica            | VT1000S  |
| Temperature controller        | Warner Instruments | CL-100   |
| Nikon Eclipse microscope      | Nikon            | E600FN   |
| Confocal microscope           | Zeiss            | LSM510   |
| Infrared-sensitive CCD camera | Hitachi          | KP-200/201 |
| Digidata 1440a interface      | Molecular Devices | N/A      |
| Item                                | Manufacturer          | Model/Part Number  |
|-------------------------------------|-----------------------|--------------------|
| Multiclamp 700B amplifier           | Molecular Devices     | N/A                |
| Borosilicate glass capillaries      | World Precision Instruments | CAT# TW150-4 |
| Sutter P-97 puller                  | Sutter Instruments    | P-97               |
| Knittel Glass coverslips            | Dutschner             | CAT# 900529        |
| Polysine slides                     | Thermoscientific      | CAT# P4981         |
| Small Animal Ventilator             | CWE                   | SAR-830/AP         |
| Cardiotachometer                    | CWE                   | CT-1000            |
**SUPPLEMENTAL DATA ITEMS**

**FIGURES AND LEGENDS**

**FIGURE S1** (Related to Figure 1)

**Figure S1** (Related to Figure 1). Firing frequency and slow afterhyperpolarization (AHP) pattern of bistable motoneurons. **A.** Voltage trace from a motoneuron displaying a pause in spiking (arrow) in response to a 7.5-s depolarizing pulse. **B-D.** Mean time-course changes of spikes in their firing frequency (B), and of afterhyperpolarizations (AHPs) in their duration (C) and amplitude (D) during tonic spiking induced by a 7.5-s supraliminal depolarizing pulse. Continuous lines are the best-fit nonlinear regression. On right, scatterplots of data collected from 28 motoneurons. (Instantaneous firing rate increases: from 15.3 ± 0.8 Hz to 24.8 ± 0.3 Hz; AHP duration decreases: from 42.3 ± 1.4 ms to 30.9 ± 0.4 ms; amplitude AHP decreases: from -19.6 ± 0.5 mV to -17.2 ± 0.2 mV, n = 28 cells). ***p < 0.001, Wilcoxon paired test. **E-F.** Amplitude (E) and duration (F) of AHPs as function of the instantaneous frequency of spikes. Continuous red lines are the best-fit regression. ***p < 0.001, Spearman correlation test. Data are mean ± SEM.
Figure S2 (Related to Figure 1). The delayed-spike frequency acceleration firing pattern is still present in lumbar motoneurons in in vivo adult mouse. A-B. Voltage traces of adult lumbar motoneuron (175 days old) in response to a subliminal (A) or liminal (B) depolarizing pulse. C. Voltage trace from the same motoneuron in response to a second depolarizing pulse subsequent to that delivered in the panel b with an interval interpulse of 5 s. Note that the delay between the onset of the pulse and the onset of firing shortened when subsequent current of same amplitude was injected. Note also that in some cases an initial burst of firing could be seen at the beginning of the pulse, followed by a pause until firing threshold was reached and the cell started to fire. Instantaneous frequency plots on top of intracellular recordings.
**Figure S3** (Related to Figure 1)

The slow membrane depolarization and the spike-frequency acceleration of bistable motoneurons are independent of Ca\(^{2+}\)-activated K\(^+\) channels and voltage-dependent Ca\(^{2+}\) channels. **A.** Superimposed voltage traces showing slow afterhyperpolarizations that follow spikes (truncated) evoked by a 3 ms current pulse before (black trace) and during 100 nM apamin (red trace). **B.** Mean peak amplitude of slow membrane depolarizations induced by a subliminal depolarizing pulse. (4.8 mV ± 0.3 mV, n = 28 cells for control *versus* 4.6 mV ± 0.7 mV, n = 7 cells under apamin). *p > 0.05, Mann–Whitney test.** C. Mean time-course changes of spikes in their firing frequency during tonic spiking induced in motoneurons by a 7.5-s supraliminal depolarizing pulse and recorded in the presence of apamin (100 nM). In means, the instantaneous firing rate grew from 18.1 ± 2.5 Hz to 32.2 ± 3.5 Hz, n = 7 cells. *p < 0.05, Wilcoxon paired test. Continuous line is the best-fit nonlinear regression. At bottom, scatterplots of data collected from the 28 motoneurons. Data
are mean ± SEM. D. Representative leak-subtracted inward currents evoked by triangular voltage ramps from -80 mV to 0 mV over 10 s in presence of TTX (n = 17 cells). E. Superimposed voltage traces recorded under TTX (1 µM) in response to depolarizing pulse before (black trace) and during (red trace) mibefradil (10 µM). On right, mean peak amplitude of slow membrane depolarizations. (4 ± 0.4 mV for control versus 4.3 ± 0.7 mV during mibefradil, n = 5 cells), p > 0.05, Wilcoxon paired test. F. Superimposed voltage traces in response to a depolarizing pulse few minutes after the internal perfusion with BAPTA (10mM) in the pipette solution. On right, mean peak amplitude of slow membrane depolarizations. (3.7 ± 0.5 mV just after establishing whole-cell access versus 3.6 ± 0.5 mV 10 minutes after establishing whole-cell access, n = 6 cells), p > 0.05, Wilcoxon paired test. Data are mean ± SEM.
Figure S4 (Related to Figure 2). Action potential properties during the firing frequency acceleration of bistable motoneurons. A–C. Mean time-course changes of spikes in their duration (A), rise slope (B) and decay slope (C) during tonic spiking induced by a 7.5-s supraliminal depolarizing pulse. Continuous lines are the best-fit regression. On right, scatterplots of data collected from 28 motoneurons. (Duration increases from 0.64 ± 0.3 ms to 0.73 ± 0.3 ms; rise slope decreases from 186 ± 9.8 mV/ms to 177 ± 9.8 mV/ms; decay slope decreases from -98 ± 4 mV/ms to -84 ± 3.4 mV/ms, n = 28 cells). **p < 0.01, ***p < 0.001, Wilcoxon paired test. D–E. Decay slope as a function of action potential duration (D), or firing frequency (E) (n = 28 cells). Each red line indicates the linear regression for one motoneuron. The rate repolarization of the action potential was linearly related to the time-course increase of both the duration of spikes (D, r²: 0.82 ± 0.03, n = 28 cells, p < 0.001; Pearson test’s correlation) and the spike-frequency acceleration (E, r²: 0.56 ± 0.04, n = 28 cells, p < 0.001; Pearson test’s correlation). Data are mean ± SEM.
Figure S5 (Related to Figure 3). The outward current in response to a prolonged depolarizing current step is potassium-mediated and stable overtime. A. Measurements of the reversal membrane potential of the current in response to prolonged depolarizing/hyperpolarizing currents of different amplitudes in presence of ZD7288 (20 µM). The continuous line represents the best-fit linear regression. The reversed polarity was $-87.71 \pm 4.28$ mV ($n = 10$ cells). B. Mean time-course changes in amplitude of the slow inactivating outward current indicated by the black vertical arrow. Values are relative to the amplitude of the first slow inactivating outward current induced after establishing whole-cell access. C. Superimposed representative outward currents in response to a depolarizing pulse recorded in extensor (black trace) and flexor (red trace) motoneurons pre-labeled with FITC conjugated Cholera toxin B. On right, mean amplitude of slow inactivating inward current indicated by the vertical arrows. ($343 \pm 51$ pA, $n = 6$ cells for extensor motoneurons versus $337 \pm 55$ pA, $n = 9$ cells for flexor motoneurons), $p > 0.05$, Mann–Whitney test. D. Superimposed outward currents in response to a depolarizing pulse in the presence of different concentrations of TEA. On right, mean amplitude of slow inactivating inward current indicated by the vertical arrows. ($182 \pm 51$ pA in 10 mM [TEA], versus $189 \pm 61$ pA in 20 mM [TEA], $n = 5$ cells). $p > 0.05$, Wilcoxon paired test. Data are mean ± SEM.
Figure S6 (Related to Figure 5). DTx-I-sensitive K+ channels promote near-threshold nonlinear firing properties in neonatal rat bistable motoneurons. A. Superimposed voltage traces in response to subliminal depolarizing pulse before (black trace) and during (red and green traces) dendrotoxin-I (DTx-I, 1 µM). B. Mean amplitude of slow depolarizations from 8 motoneurons as function of the membrane potentials before DTx-I (black) and during DTx-I (red). Continuous lines are the best-fit linear regression. Mean peak amplitude \(4.27 \pm 1.05\) mV for control; \(1.26 \pm 0.5\) mV during DTx-I, \(n = 8\) cells). \(* * * p > 0.001\) Wilcoxon paired test. C-D. Representative voltage traces in response to a liminal (C) or supraliminal (D) depolarizing pulse before (black trace) and during (red trace) DTx-I. Instantaneous frequency plots on top of intracellular recordings. E) Mean time-course changes in instantaneous firing frequency from all recorded motoneurons before (black) or during
DTx-I (red) in response to a supraliminal depolarizing pulse (n = 8 cells). Instantaneous firing frequency changes from $25.08 \pm 0.47$ Hz to $30.37 \pm 0.09$ Hz before DTx-I and from $34.34 \pm 0.59$ Hz to $31.38 \pm 0.59$ Hz during DTx-I). $p > 0.05$, **$p < 0.01$, Wilcoxon paired test. F-G. Representative voltage traces before (black trace) and during DTx-I (red and green traces) in response to subliminal (F) or liminal (G) oscillatory currents (1 Hz). On right of the panel F, mean peak amplitude increases of membrane oscillations. ($4.36 \pm 0.29$ mV for control versus $0.27 \pm 0.12$ mV during DTx-I, n = 7 cells. **$p < 0.01$, Wilcoxon paired test. All recordings are performed under kynurenic (1.5 mM). Note that under DTx-I a bias current was used to maintain the pre-pulse membrane potential at the holding potential fixed in control condition. For a direct comparison current pulses were also adjusted to reach the same level of depolarization at the onset of the pulse as that for control (red trace in F). Note a same current amplitude as in control was also injected (green trace in F). Data are mean ± SEM.
**Figure S7** (Related to Figure 6)

**A.** Voltage dependence of steady activation $k_\infty$ (black trace) and inactivation $l_\infty$ (grey trace). **B-C.** Time constants of steady activation (B) and inactivation (C). Dotted line in panel C the half-reduced inactivation time constant. **D.** Time-course of outward current evoked in a single compartment model of the membrane populated by Kv1.2 potassium and passive leak channels (conductivities $g_{Kv1.2} = 0.25$ S/cm$^2$ and $g_{leak} = 0.05$ S/cm$^2$, respectively) by application of 8-sec voltage steps of increasing intensity (-60 to -20 mV, increment 10 mV). **E.** Current responses to the same as in panel D -30 mV voltage step in cases of the original $t_i$ (red trace) and half-reduced $t_i/2$ inactivation time-constants (black trace). **F-G.** The duration of the pre-firing depolarization became about two-times shorter if the initial time constant inactivation (F) of Kv1.2 channels was reduced by 50% (G).
TABLE S1 (Related to Figure 4 and Figure S5)

| Condition                        | Peak outward currents (pA)                                                                 |
|----------------------------------|-------------------------------------------------------------------------------------------|
| Control vs Nifedipine            | 1297 ± 63.61 vs 929 ± 102* (n=6, * p<0.05, Wilcoxon t-test)                                |
| Control vs Stromatoxin           | 985.7 ± 74.82 vs 911.3 ± 68.9 (n=12, ns, Wilcoxon t-test)                                 |
| Control vs 4-AP                  | 762.2 ± 86.02 vs 491.1 ± 47.9* (n=6, * p<0.05, Wilcoxon t-test)                            |
| Control vs DTX-I vs wash         | 992.3 ± 37.91 vs 672.6 ± 23.74*** vs 915.8 ± 34.59 (n=8, *** p<0.001, One-way ANOVA)      |
| Control vs DTX-K                 | 1261.1 ± 129.6 vs 1237.4 ± 117.4 (n=6, ns, Wilcoxon t-test)                               |
| Control vs TsTX vs wash          | 894.8 ± 111.6 vs 567 ± 80.42** vs 734.8 ± 174.9 (n=8, ** p<0.01, One-way ANOVA)           |
| Extensor vs Flexor               | 1156.2 ± 74.75 vs 1122.2 ± 110.9 (n=6 et 7, ns, Mann-Whitney t-test)                      |
| TEA (10mM) vs TEA (20mM)         | 1258.1 ± 286.2 vs 1266.8 ± 270.1 (n=5, ns, Wilcoxon t-test)                               |

**Peak outward currents of lumbar motoneurons in different conditions.** Values are mean ± s.e.m.; n = number of cells. All parameters have been recorded in voltage-clamp mode.
# TABLE S2 (Related to Figure 5 and Figure S6)

|                  | Control (kynurenone) | DTX-I (1µM) (n=8) | Control (kynurenone) | TsTX (1µM) (n=7) |
|------------------|----------------------|-------------------|----------------------|------------------|
| \( R_{\text{in}}, \text{M\Omega} \) | 26.77 ± 2.26         | 30.11 ± 2.52 *    | 32.23 ± 4.16         | 33.62 ± 3.42     |
| AP amplitude, mV | 60.15 ± 1.54         | 55.94 ± 2.23 *    | 64.77 ± 0.65         | 62.33 ± 2.61     |
| AP duration, ms  | 0.61 ± 0.02          | 0.64 ± 0.02       | 0.72 ± 0.05          | 0.68 ± 0.05      |
| AP threshold, mV | -57.24 ± 1.12        | -59.24 ± 1.52 *   | -55.61 ± 1.39        | -55.34 ± 2.11    |
| Rheobase, pA     | 962.5 ± 91.49        | 850.1 ± 82.38     | 983.33 ± 134.6       | 933.33 ± 143     |
| First spike latency, s | 3.44 ± 0.41   | 0.62 ± 0.28**     | 2.73 ± 0.52          | 0.65 ± 0.38**    |
| Holding, pA      | -48.90 ± 13.76       | -169 ± 47.26*     | -62.72 ± 22.65       | -197.9 ± 35.09*  |

Passive and active membrane properties of motoneurons before or during the application of dendrotoxin-I (DTX-I) or Tityustoxin (TsTX). Values are mean ± SEM.; n, number of cells; \( R_{\text{in}} \), input resistance; AP, action potential. Statistical significance was assessed by Wilcoxon test. *\( p < 0.05 \), **\( p < 0.01 \). All the parameters have been recorded in current clamp mode.