NMDA induces persistent inward and outward currents that cause rhythmic bursting in adult rodent motoneurons

Marin Manuel,1 Yaqing Li,2 Sherif M. ElBasiouny,1 Katie Murray,2 Anna Griener,2 C. J. Heckman,1,3 and David J. Bennett2

1Department of Physiology, Northwestern University Feinberg School of Medicine, Chicago, Illinois; 2Centre for Neuroscience, University of Alberta, Edmonton, Alberta, Canada; and 3Departments of Physical Medicine and Rehabilitation, Physical Therapy and Human Movement Science, Northwestern Feinberg School of Medicine, Chicago, Illinois

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Manuel M, Li Y, ElBasiouny SM, Murray K, Griener A, Heckman CJ, Bennett DJ. NMDA induces persistent inward and outward currents that cause rhythmic bursting in adult rodent motoneurons. J Neurophysiol 108: 2991–2998, 2012. First published September 12, 2012; doi:10.1152/jn.00518.2012.—N-Methyl-D-aspartate (NMDA) receptors are of critical importance for locomotion in the developing neonatal spinal cord in rats and mice. However, due to profound changes in the expression of NMDA receptors in development between the neonatal stages and adulthood, it is unclear whether NMDA receptors are still an important component of locomotion in the adult rodent spinal cord. To shed light on this issue, we have taken advantage of recently developed preparations allowing the intracellular recording of adult motoneurons that control the tail in the sacrocaudal spinal cord of adult mice and rats. We show that in the adult sacrocaudal spinal cord, NMDA induces rhythmic activity recorded on the ventral roots, often coordinated from left to right, as in swimming motions with the tail (fictive locomotion). The adult motoneurons themselves are intrinsically sensitive to NMDA application. That is, when motoneurons are synchronically isolated with TTX, NMDA still causes spontaneous bursts of rhythmic activity, depending on the membrane potential. We show that these bursts in motoneurons depend on an NMDA-mediated persistent inward current and are terminated by the progressive activation of a persistent outward current. These results indicate that motoneurons, along with the central pattern generator, can actively participate in the production of swimminglike locomotor activity in adult rodents.

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N-METHYL-D-ASPARTATE (NMDA) receptors (NMDARs) are a family of glutamate receptors widely expressed in the central nervous system. NMDARs exert a central role in numerous physiological processes, such as synaptic plasticity. In the spinal cord, NMDARs are likely involved in locomotion, as NMDA is a critical compound in the drug cocktails used to initiate fictive locomotion in isolated neonatal mouse or rat spinal cords. Locomotion requires a precise spatiotemporal activation of muscle groups to produce the right sequence of movements. The generation of locomotion in vertebrates is mainly attributed to a population of spinal interneurons called the central pattern generator (Burke et al. 2001; Grillner and Wallen 1985; Kiehn and Butt 2003; Rossignol 1996; Wallen and Grillner 1985), the role of which is to generate the basic rhythm and to send a synaptic drive to the various motor pools (McCrea and Rybak 2008). The drugs used to elicit locomotor activity in vitro (fictive locomotion) likely act on these interneurons (Goulding et al. 2002; Jessell 2000; Kiehn and Butt 2003; Lanuza et al. 2004; Wenner et al. 2000). Yet, several studies have shown that, in lamprey, frogs, turtles, and neonatal rodents, NMDA can have a direct effect on the motoneurons themselves (del Negro et al. 1999; Guertin and Houngsaard 1998; Hsiao et al. 2002; Kim and Chandler 1995; MacLean et al. 1997; Rioult-Pedotti 1997; Wallen and Grillner 1985). In these animals, NMDA can trigger slow oscillations of the membrane potential independently of synaptic inputs onto motoneurons.

The role of NMDARs in adult mammalian motoneurons remains, however, uncertain. In rodents, NMDARs are abundant during the 1st postnatal week throughout the spinal cord but are dramatically reduced thereafter (Kalb et al. 1992; Palecek et al. 1999). For example, the monosynaptic Ia excitation to motoneuron transitions from having a significant NMDA component in the neonate to virtually none in the adult (Engberg et al. 1993). Nonetheless, some NMDARs are still present in the adult spinal cord (Monyer et al. 1994; Piehl et al. 1995; Tolle et al. 1993; Wee et al. 2008). Moreover, in the brain stem, administration of NMDA has been shown to depolarize motoneurons that control eye movements (Durand 1991, 1993). The goal of this paper is to investigate the direct action of NMDARs on adult motoneurons of rodents. To do so, we have taken advantage of the recently developed in vitro sacral cord preparation (Bennett et al. 2001; Jiang and Heckman 2006), which allowed us to perform intracellular recordings of the motoneurons innervating the tail muscles of both adult mice and rats.

MATERIALS AND METHODS

Intracellular recordings were made from motoneurons in the sacrocaudal spinal cord of adult mice (age 30–50 days old) and rats (age 80–120 days). All experimental procedures were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee (rat experiments) and the Northwestern University Institutional Animal Care and Use Committee (mouse experiments). Procedures were identical in rat and mouse experiments.

Surgery. Animals were deeply anesthetized with urethane (0.18 mg/100 g), and the spinal cord caudal to the T12 vertebrae was transferred to a dissection dish containing oxygenated modified artificial cerebral spinal fluid (mACSF) at room temperature (20–21°C). Following a 1-h resting period in mACSF, the cord was transferred to a recording chamber, where it was submerged in normal ACSF
N-methyl-D-aspartate (NMDA)-induced bursting activity on ventral roots. A: root recording from a mouse spinal cord showing the rectified and integrated activity on the right ventral root S2 (top trace) and the raw electrical activity recorded on ventral root S2 (bottom trace). The left part of the trace illustrates the control condition with no NMDA present in the bath. Little to no spontaneous activity was visible in those conditions. The middle part shows the bursts that appeared on the ventral root S2 when NMDA was applied to the bath. When the NMDA was washed out (right part), the activity returned to its initial quiescent state. B: recordings from the left and right S4 ventral roots of a rat spinal cord. In presence of NMDA (75 µM), bursts of activity could be observed on the roots, alternating from left to right (vertical dashed lines demark leftside bursts) and increasing with time after application (application 3 min before recording). Eventually, tonic synchronous activity also emerged (right of plot).

flowing at 3–6 ml/min and maintained at 25°C. The cord was loosely supported on a nappy paper mesh and secured by passing insect pins through lateral vasculature and connective tissue and into a silicone elastomer (Sylgard) base below the nappy paper. For intracellular recording, and not ventral root recording, the ventral surface was oriented upward and the dorsal surface of the cord was glued (with cyanoacrylate) to the nappy paper to stabilize further the cord to enable long-term intracellular recordings.

**Solutions.** The normal ACSF had the composition, in mM, 122 NaCl, 24 NaHCO3, 3 KCl, 2.5 CaCl2, 1 MgSO4, and 12 glucose in distilled water, bubbled with 95% O2-5% CO2, pH 7.4. An mACSF was used during dissection and recovery to prevent excitotoxic injury. The mACSF composition was, in mM, 118 NaCl, 24 NaHCO3, 3 KCl, 1.5 CaCl2, 1.3 MgSO4, 25 glucose, 1.4 NaH2PO4, 5 MgCl2, and 1 kynurenic acid (McQuiston and Madison 1999). NMDA (Sigma-Aldrich) and tetrodotoxin (TTX-citrate; Alomone) were added to the ACSF as described in the text.

**Intracellular and extracellular recording.** Intracellular recordings were obtained using sharp intracellular electrodes filled with either 3 M KCl or a mixture of 1 M K-acetate and 1 M KCl and beveled to a resistance of 25–30 MΩ using a rotary beveller (BV-10; Sutter Instruments). A Stepper Motor (Model 2660 Micropositioner; David Kopf Instruments) was used to advance the electrodes vertically into the ventral horn, and intracellular recordings from motoneurons were made with an AxoClamp 2B intracellular amplifier (Axon Instruments) running in discontinuous current clamp (switching rate 5–8 kHz, output bandwidth 10 kHz) or discontinuous single-electrode voltage clamp (gain 0.8–2.5 nA/mV) modes and sampled at 6.7 kHz with a Clampex system (Axon Instruments) or at 20 kHz with a Spike2/1401plus system (Cambridge Electronic Devices). The ventral roots were wrapped around Ag/AgCl wire electrodes and sealed with grease, which allowed for antidromic stimulation identification of motoneurons. Motoneurons with a resting potential below −60 mV and antidromic spike overshoot >0 mV were considered healthy and used for recording. In some animals, we also recorded from the ventral roots on the Ag/AgCl electrodes (ventral root extracellular recording).

**RESULTS**

**NMDA-induced bursting.** As a first step, we examined whether bath application of NMDA could alter the activity of adult mouse and rat motoneurons by recording the global activity of the motor pools in the ventral roots S2–S4 that innervate the tail. As illustrated in Fig. 1, administration of NMDA (30–80 µM in both mice and rats) usually induced a regular bursting in the sacral ventral roots that began within a few minutes and persisted for several minutes (n = 9/13 mouse spinal cords; n = 9/12 rat cords). In all cases, whether or not bursts could be observed, a significant increase of tonic activity was observed on all ventral roots recorded, and oscillations superimposed on this tonic activity could sometimes be seen lasting from minutes to hours. Washout of NMDA eliminated this regular bursting and tonic activity within a few minutes (data not shown). Bursting activity was often organized in an alternating left-to-right manner (Fig. 1B), which, in the intact animal, would move the tail side-to-side in a swimming motion, as seen in the neonatal rat (Delvolve et al. 2001). This organized bursting became less clear once the tonic activity emerged (Fig. 1B, right).

**Bursts are intrinsic to motoneurons.** To investigate the role of motoneurons in NMDA-induced bursts, we performed intracellular recordings in 11 mouse and 15 rat sacrocaudal motoneurons. A typical example is illustrated in Fig. 2. In this mouse motoneuron, bath application of NMDA (75 µM) induced, within 1 min, a steady depolarization that brought the motoneuron to threshold. After a minute of slow, long, repeated bursts of firing, the motoneuron began firing tonically. Using a bias current to drive the membrane potential to a more hyperpolarized value, we observed shorter bursts −1 s in duration, each of which started spontaneously. These bursts repeated about every 6 s, as long as the cell was held hyperpolarized (Fig. 2, right). Overall, we observed similar spontaneous bursts in 8 of the 11 mouse and 14 of 15 rat motoneurons (73 and 93%, respectively) recorded. However, they were...
sometimes masked by the global depolarization of the motoneuron and required various amounts of hyperpolarizing current to be revealed. Generally, we found that motoneuron bursting required NMDA doses >30 μM, similar to bursting in ventral roots.

Two opposite hypotheses can be proposed to explain these bursts. Either they are created by premotor interneurons impinging on the motoneurons that become rhythmically active in presence of NMDA or the bursts are intrinsic to the motoneurons. In the former hypothesis, bursts of synaptic activity should remain when the motoneuron is hyperpolarized below the activation threshold for the NMDARs, and the bursts should disappear in presence of TTX. In the latter hypothesis, hyperpolarization of the membrane should inactivate the intrinsic current(s) responsible for the bursts, and, in presence of TTX, the underlying depolarization should remain visible.

We found that increasing hyperpolarization progressively reduced the burst frequency and duration until bursts disappeared completely at the most hyperpolarized levels (n = 7/7 mouse cells and 15/15 rat cells). The motoneuron illustrated in Fig. 3 showed spontaneous bursts at rest (without bias current). Hyperpolarizing the motoneuron with −0.4 nA of bias current slightly decreased the frequency and duration of the bursts. Further hyperpolarization decreased the frequency and duration of the bursts even more. For bias currents more than −1.5 nA, the bursts disappeared completely, and no subthreshold oscillations, indicative of bursts of synaptic activity, could be observed. This observation is consistent with an intrinsic origin for the NMDA-induced bursts in motoneurons. Importantly, these intracellular data were collected from cords that had glue on their dorsal surface (unlike during ventral root recording) to hold the cord to the bottom of the dish (nappy paper) to stabilize mechanically the intracellular recordings. This likely did not favor interneuron-mediated NMDA bursting because of the poorer oxygenation of the glued dorsal surface. Indeed, when we did not use the glue on the cord, then subthreshold oscillations could be seen in NMDA under hyperpolarized conditions (n = 4/4 cords; data not shown), suggesting additional involvement of interneurons, although the recordings did not last long because of mechanical instability (see DISCUSSION).

To confirm the involvement of motoneurons in bursting, we applied TTX (1 μM) after the initiation of the NMDA bursts to see whether they persisted. A typical example is shown in Fig. 4. In this motoneuron, bath application of NMDA (30 μM) initiated spontaneous bursts of activity. Adding TTX (1 μM) in addition to the NMDA blocked the spikes, as expected, but failed to block the underlying depolarizations: waves of spontaneous depolarization remained, followed a few seconds later by a sharp return to baseline. The same behavior was observed in three of the four mouse cells tested with TTX and six of the six rat cells tested.

**Ionic origin of the NMDA-induced oscillations.** To investigate the origin of the NMDA-induced bursting, we performed voltage-clamp experiments in 15 adult rat motoneurons in the presence of TTX. With TTX, both the synaptic input to motoneurons (spike mediated) and the sodium component of the persistent inward current (Na PIC) were blocked, but there remained a Ca PIC. This Ca PIC was seen in voltage clamp during a slow ramp to produce a downward deflection in current (thick line in Fig. 5) relative to the extrapolated leak current (thin line). This Ca PIC was activated at −55 ± 7 mV (V0n; n = 6), near the usual sodium spike threshold but significantly above the resting potential (−70 ± 7 mV, n = 6; P < 0.05), as previously described (Harvey et al. 2006; Li et al. 2004). On average, the Ca PIC amplitude was −0.8 ± 0.4 nA (measured at initial peak in current). Importantly, the Ca PIC remained activated even when the voltage was ramped back down below where it was initiated (V0n) and thus produced a clear hysteresis, characteristic of this current.

After application of NMDA (75–100 μM), a large and completely different PIC emerged that we term the NMDA PIC (Fig. 5B). This current was initiated at a very hyperpolarized potential (−76 ± 7 mV, V0n,NMDA, n = 6), approximately at the resting membrane potential before NMDA (not significantly different from rest) and significantly lower than the Ca PIC onset voltage before NMDA (P < 0.05). With NMDA present, the motoneurons had to be held with a negative bias current of about −2 nA to prevent the NMDA PIC activation because NMDA significantly reduced the overall PIC onset current (I0n) from +1.0 ± 0.7 nA before NMDA (Ca PIC) to −2.1 ± 1.1 nA in NMDA (NMDA PIC onset current). The NMDA PIC amplitude was −2.2 ± 0.9 nA (initial peak PIC), nearly three times larger than the Ca PIC before NMDA (P < 0.05). Unlike the Ca PIC, the NMDA PIC tended to inactivate with time and lacked inward current hysteresis. That is, a few seconds after the NMDA PIC was activated, it slowly decreased and reversed to a significant persistent outward current (POC; +0.6 ± 0.3 nA, measured relative to the leak line after PIC end as in Fig. 5B), which remained activated as the voltage was ramped back to rest. Before NMDA, no significant POC existed (0.1 ± 0.2 nA, measured at the same voltage). These NMDA PICs and POCs were readily distinguished from the Ca

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**Fig. 3.** Effect of hyperpolarization on the NMDA-induced bursts. Intracellular recording was from a mouse motoneuron. On continuous bath application of NMDA 40 μM (top line), this motoneuron was driven to more and more hyperpolarized potentials with a bias current (intensity written on top of the trace). As the motoneuron got more and more hyperpolarized, the frequency and duration of the bursts decreased, and they even stopped completely at −1.5 nA of bias current.

**Fig. 4.** NMDA-induced bursts persist in presence of TTX. This mouse motoneuron exhibited spontaneous bursts of activity in presence of NMDA (2nd line from the top). Adding TTX (1 μM; top line) in addition to the NMDA stopped the firing but not the underlying depolarizations causing the bursting, as indicated by the waves of depolarization still visible on the right.

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Fig. 5. Currents responsible for the NMDA-induced bursting. A: Ca persistent inward current (PIC) in a rat motoneuron recorded in presence of TTX (1 μM), activated by slowly increasing the membrane potential under voltage clamp (top trace), and quantified at its initial peak, where it produced a downward deflection in the recorded current (at arrow) relative to the leak current (thin line). B: voltage-clamp recording of a rat motoneuron in presence of NMDA (100 μM) and TTX (1 μM). Same organization as A. In these conditions, the Ca PIC is hidden by a larger NMDA PIC (downward arrow), and, at the end of the voltage ramp, a persistent outward current (POC; upward arrow) becomes visible. C: voltage-clamp recording of a rat motoneuron in presence of TTX (1 μM) and isradipine (15 μM). Same organization as A. Isradipine is used to eliminate Ca PIC and NMDA PIC interactions by blocking the Ca PIC. As a consequence, the voltage ramp gave a linear current response. D: voltage-clamp recording of a rat motoneuron in presence of TTX (1 μM), isradipine (15 μM), and NMDA (100 μM). Same organization as A. Even when the Ca PIC is blocked by isradipine, clear NMDA PIC and POC are visible on this recording. E: response (bottom thick trace) of a rat motoneuron to a 10-mV voltage step from −77 to −67 mV (top trace). The NMDA PIC is initially visible as a downward deflection in the current trace (downward arrow). The net inward current then decreased continuously after this peak due to the progressive activation of the POC (upward arrow). F: current-clamp recording of the same motoneuron as in E showing the NMDA-mediated depolarization (plateau), which spontaneously turns off and reveals an hyperpolarization phase due to the POC.
PIC in most motoneurons because they typically were much larger and lower threshold than the Ca PICs and indeed usually masked any sign of Ca PIC activation in the presence of NMDA (Fig. 5, A and B). However, in 1 cell, we saw the Ca and NMDA PICs come on at separate potentials (as seen by 2 negative slope regions in the current response on the upward ramp; data not shown).

To eliminate Ca PIC and NMDA PIC interactions, we investigated the action of NMDA in isolation in five rat motoneurons that had the Ca PIC blocked with isradipine (15 μM) and synaptic input and Na PIC blocked with TTX (Fig. 5C). In these motoneurons, before NMDA application, a voltage ramp gave a linear current response, confirming the block of the Ca and Na PICs (n = 5/5 cells), as previously detailed (Li and Bennett 2003). Application of NMDA (75–100 μM) produced a low-threshold and large PIC (NMDA PIC; Fig. 5D) with the same asymmetric characteristics followed by a POC, as seen before the Ca PIC block. That is, the NMDA PIC was activated at −71 ± 5 mV, again near the predrug resting potential (−69 ± 10 mV), and required a negative bias current to prevent activation (activation current −0.9 ± 1.5 nA). This NMDA PIC was on average −1.7 ± 0.9 nA (initial peak) and not significantly different from the NMDA PIC measured without the Ca PIC block, confirming that the NMDA PIC dominates over the Ca PIC. Again, the NMDA PIC decreased slowly with time and was replaced by significant net outward current (POC) that could always be seen on the downward ramp of the voltage-clamp protocol, which was on average +0.7 ± 0.3 nA.

To investigate further the timing of these NMDA currents, we initially held the motoneurons at a potential below the resting potential to prevent the NMDA PIC activation and then applied steps in voltage (Fig. 5E; n = 5). Steps over the resting potential initiated the NMDA PIC with a time to peak current of ~0.5 s. The net inward current then decreased continuously after this peak, suggesting the onset of a POC that increased with time. This POC could be seen in isolation when the voltage step was terminated, and then lasted >2 s, in all cells tested (n = 5/5). It was clear that these currents caused plateau potentials and membrane oscillations when the cells were not under voltage-clamp control (Fig. 5F). That is, in these same cells, releasing the voltage clamp and removing all bias current led to a rapid depolarization (plateau), mediated by the NMDA PIC (at its activation threshold). When a small negative bias current was applied, the membrane potential oscillated slowly. That is, a plateau was again activated but then slowly decreased to a point where there was a sudden termination of the plateau and the onset of an afterhyperpolarization that lasted for a few seconds, which was mediated by the POC (at same voltage). At the end of this POC-mediated afterhyperpolarization, the plateau was reactivated and the whole process continued, leading to the observed spontaneous oscillations (Fig. 6A; n = 5/5 cells). When additional depolarizing bias current was applied during these oscillations, the NMDA plateau lasted longer (Fig. 6B), thus explaining the longer bursts of firing seen at depolarized levels without TTX (Fig. 3).

**DISCUSSION**

We demonstrate here that in the adult rodent spinal cord, the motoneurons innervating the muscles of the tail are sensitive to application of NMDA. NMDA induces intrinsic bursts of activity in these motoneurons, which suggests that they can take an active role in the production of the rhythmic activity required for swimming. In general, our results support the possibility that locomotion in the adult state shares the NMDA-driven behavior seen in the neonatal preparation (Kiehn et al. 2000; MacLean et al. 1997; Schmidt et al. 1998; Wallen and Grillner 1987).

**Ionic conductances responsible for the bursts.** We showed that the bursts and the underlying waves of depolarization can be elicited in absence of all synaptic activity since they remained in presence of TTX. They are therefore partly due to an intrinsic property of the motoneurons. The bursts are reminiscent of plateau potentials classically described in other motoneurons and caused by Ca PICs. However, Ca PICs are not very strong in our experimental conditions where the cords are acutely removed from normal rats and mice, and thus levels of serotonin and norepinephrine are low (M. Manuel, S. M. ElBasiouny, and C. J. Heckman, unpublished observations; Li and Bennett 2003; Murray 2010), and, furthermore, NMDA-induced bursting persisted in a block of Ca PICs and Na PIC. Thus these bursts are likely mediated by an NMDA current from the NMDARs themselves.

The NMDA current, because of its magnesium block, has a voltage dependence and thus behaves like the Ca and Na PICs described in motoneurons (Flatman et al. 1983; MacDonald et al. 1982; Mayer et al. 1984; Nowak et al. 1984). Voltage-clamp experiments presented here show that after NMDA application, motoneurons exhibit a new PIC that is readily distinguishable from the Na and Ca PICs. This PIC (NMDA PIC) is the current sustaining the depolarizing phase of the slow waves of depolarization (NMDA Plateau). Activation of the NMDA PIC is followed by the activation of an outward current (POC), which is the current responsible for the spontaneous termination of the NMDA plateaus. We suggest that this POC was produced by the calcium entering the cell through the NMDAR and slowly activating a calcium-activated current (SK) that persisted until the calcium was sequestered, as this was proven to be the mechanism responsible for burst termination in the lamprey (el Manira et al. 1994; Wallen and Grillner 1987), although we cannot rule out the role of Na\(^{+}\)-K\(^{+}\)-ATPase pump currents (del Negro et al. 1999; Hsiao et al. 2002; Kim and Chandler 1995).

**Functional consequences.** Our results show that, under the right set of conditions, activation of NMDARs on adult mo-

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**Fig. 6.** Voltage dependence of the NMDA-induced plateaus. Current-clamp recording of a rat motoneuron in presence of isradipine (15 μM), TTX (1 μM), and NMDA (100 μM) is shown. This motoneuron exhibited spontaneous NMDA plateaus at rest, which terminated and restarted repeatedly. When the cell was depolarized by injection of a bias current (0.5 nA), the plateaus lasted longer.
töneurons can lead to their direct recruitment, including produc-
tion of rhythmic bursts of activity. The motoneurons of the
sacral region of the spinal cord are connected to the
muscles of the tail (Brink and Pfaff 1980; Grossman et al.
1982; Masson et al. 1991), and although we did not identify
whether the recorded motoneurons were connected to extensor
or flexor muscle groups, it has been shown that bursts of
activity in sacral motoneurons of neonatal rats are able to
elicit left-to-right abductions of the tail, similar to during
swimming (Delvolve et al. 2001). Thus our results are consis-
tent with the NMDA-mediated input to motoneurons occurring
during locomotion. A fundamental role of NMDAR activation
on motoneurons in the adult state during rhythmic activity is
supported by the recent study of Enríquez Denton et al. (2012).
This study showed that block of NMDARs markedly sup-
pressed respiratory oscillations in phrenic motoneurons. Al-
though this study was done using an in vivo preparation
(decerebrate cat), this block was achieved via injection of the
drug from an intracellular microelectrode, showing that the
NMDARs blocked were highly likely to be on the motoneurons
themselves. There may, however, also be sensory inputs that
activate NMDARs on motoneurons. Ia monosynaptic excit-
atory postsynaptic potentials (EPSPs) are not mediated by
NMDARs to any significant degree (Engberg et al. 1993), but
Brownstone et al. (1994) demonstrated that EPSPs generated
by stimulation of flexion reflex afferents undergo a voltage-
dependent amplification in an anesthetized preparation where
there is unlikely to be any contribution of amplification from
Ca and Na PICs (Hultborn et al. 2003; Lee and Heckman 2000;
Svitriski and Hounsgaard 1998). Thus the voltage-dependent
amplification of this sensory input may involve NMDAR
activation, but this possibility has not yet been tested.

Although our data clearly demonstrate that NMDA can
induce rhythmic alternation in motoneurons, the relative con-
tribution of NMDA oscillations in motoneurons compared with
interneurons is harder to assess. Considering that motoneurons
are not electrically or chemically coupled in adults rodents
(Chang et al. 1999; Kandler and Katz 1995; Walton and
Navarrete 1991), it is likely that any intrinsic oscillatory
activity in a single motoneuron will not be synchronized with
the activity of other motoneurons, and thus NMDA oscillations
in motoneurons cannot by themselves initiate the organized
locomotor-like activity of motoneurons pools seen in our
ventral root recordings. Thus interneuron oscillations must
initiate the synchronous activation of a given motoneuron pool
(say, leftside) and coordinate the transition to other pool
activity (left-to-right alternations). The NMDA PIC currents
in the motoneurons would then contribute to amplifying and
prolonging this underlying interneuron drive to the motoneuron
pools. Likewise, NMDA POCs in motoneurons would help
terminate bursts during locomotor behavior. Also, excess
NMDA would lead to tonic activation of NMDA POCs on
motoneurons and explain the tonic activity seen to follow the
rhythmic activity in motoneuron pools.

Our finding that NMDA-induced oscillations in motoneu-
crons can sometimes be eliminated by hyperpolarizing a mo-
toneuron does not rule out the involvement of interneurons
because the experimental conditions that we used to make
stable intracellular motoneuron recordings do not favor in-
terneuron activity, as the spinal cord was glued on its dorsal
surface, unlike during ventral root recordings. We have found
that interneuron-mediated NMDA oscillations can be seen in
motoneurons under hyperpolarized conditions when we reduce
or eliminate the glue, although recordings are not stable, thus
verifying this experimental limitation.

The electrical behavior of the adult motoneuron has proven
to be unexpectedly complex. At least three distinct states have
now been demonstrated. First, for many years, the properties of
motoneurons were assumed to conform to their behavior in
anesthetized preparation when driven by ionotropic inputs
(reviewed by Binder et al. 1996). Perhaps this relatively unex-
citable state can be considered the base state of the motoneuron.

The discovery of Ca PICs (Schwindt and Crill 1977) and
their neuromodulation by the brain stem via axons that release
ether serotonin or norepinephrine (Holstege and Kuyper
identified a new and highly excitable state of motoneurons
controlled by neuromodulators (2nd state, neuromodulatory
state). Work in several laboratories established that PICs are
likely to be a standard component of motoneuron behavior in
normal behavior in both animals and humans (reviewed in
Serotonin and norepinephrine also depolarize resting mem-
brane potentials and hyperpolarize spike thresholds (Binder
et al. 1996; Powers and Binder 2001) so that these brain-stem
neuromodulatory systems have the capacity to alter greatly the
net input-output gain of motoneurons (Heckman et al. 2008b;
2009; Hultborn et al. 2004). The PIC effects are in fact so
strong that a major role of inhibitory inputs may be to control
excess motoneuron excitability by deactivating the PIC, pro-
viding a focused control to oppose the diffuse and widespread
effects of the brain-stem neuromodulatory system (Bennett
et al. 1998; Heckman et al. 2008a; Hyngstrom et al. 2007).
Inhibition of the PIC may often be coupled to excitation in a
push-pull fashion to achieve this goal (Bennett et al. 1998;
Johnson et al. 2012). Equally important, strong neuromodula-
tory inputs originate within the spinal cord (Power et al. 2010),
further contributing to this neuromodulatory state, with for ex-
ample the motoneuron afterhyperpolarization controlled by
the cholinergic inputs activating the large “C” synaptic boutons
on motoneurons (Miles et al. 2007; Zagoraiou et al. 2009).

The NMDA-driven motoneuron activity demonstrated in
the present paper is yet another state of the motoneuron (3rd state).
This NMDA state is inherently oscillatory and thus seems
well-suited to locomotion. The input-output properties of
motoneurons thus have the potential to be reconfigured for dif-
ferent motor behaviors, depending on the mixture of inputs from
brain-stem neuromodulatory centers, and spinal neuromodula-
tory and NMDA systems. Many questions remain about how
these systems interact in generating the wide range of normal
motor behaviors. One particularly important question is how
these systems change in pathological states. There is remark-
able recovery of Ca PIC activity after spinal cord injury via
constitutive activity of receptors for 5-HT and NE (Murray et
al. 2010, 2011; Rank et al. 2011), but changes in NMDA inputs
require further study.

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GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
M. Manuel and S. M. ElBasioiny collected and analyzed the mouse data. Y. Li, A. Griefer, K. Murray, and D. J. Bennett collected and analyzed the rat data. M. Manuel, C. J. Heckman, and D. J. Bennett wrote the article.

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