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

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MM and SEM collected and analyzed the mouse data. YL, AG, KM and DJB collected and analyzed the rat data. MM, CJH and DJB wrote the article.

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Running Head

NMDA-induced bursting in adult rodent motoneurons
Abstract

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 synaptically isolated with tetrodotoxin (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 swimming-like locomotor activity in adult rodents.

Keywords

Spinal cord; Fictive locomotion; Persistent Inward Current (PIC); Persistent Outward Current (POC)
Introduction

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 spatio-temporal 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 (CPG) (Burke et al. 2001; Grillner and Wallen 1985; Kiehn and Butt 2003; Rossignol 1996; Wallen and Grillner 1985) whose role 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 Hounsgaard 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 first 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 NMDA receptors 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 brainstem, 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.

Material 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 by 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 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 further stabilize the cord to enable long-term intracellular recordings.

**Solutions**

The normal ACSF had the following 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 and pH 7.4. A modified ACSF (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 (DCC; switching rate 5–8 kHz, output bandwidth 10 kHz) or discontinuous single-electrode voltage clamp (SEVC; 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, Inc). 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 if 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 Figure 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-right manner (Figure 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 (right side of Fig 1B).

Bursts are intrinsic to motoneurons

To investigate 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 Figure 2. In this mouse motoneuron, bath application of
NMDA (75 µM) induced, within one minute, 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 about one second in duration, each of which started spontaneously. These bursts repeated about every 6 seconds, as long as the cell was held hyperpolarized (right side of Fig 2). 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 over 30 µM, similar to bursting in ventral roots.

Two opposite hypotheses can be proposed to explain these bursts. Either they are created by pre-motor 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 NMDA receptors, 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 Figure 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 sub-threshold 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 mechanically stabilize 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; not shown), suggesting additional
involvement of interneurons, though 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 if they persisted. A typical
example is shown in Figure 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 6 of the 6 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 Figure 5), relative to the
extrapolated leak current (thin line). This Ca PIC was activated at $-55 \pm 7$ mV ($V_{on}$,
$N = 6$), near the usual sodium spike threshold, but significantly above the resting
potential ($-70 \pm 7$ mV, $N = 6$, $p < 0.05$), as previously described (Harvey et al. 2006;
Li et al. 2004). On average the Ca PIC amplitude was $-0.8 \pm 0.4$ 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 ($V_{on}$) and thus
produced a clear hysteresis, characteristic of this current.

After application of NMDA (75—100 uM) a large and completely different
persistent inward current emerged that we term the NMDA PIC (Figure 5B). This
current was initiated at a very hyperpolarized potential ($-76 \pm 7$ mV, $V_{on}$ NMDA,
$N = 6$), approximately at the resting membrane potential prior to NMDA (not
significantly different from rest), and significantly lower than the Ca PIC onset
voltage prior to 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 (I_{on})
from +1.0 ± 0.7 nA prior to 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 prior to 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 Figure 5B), which remained activated as the voltage
was ramped back to rest. Prior to NMDA no significant POC existed (0.1 ± 0.2 nA,
measured at the same voltage). These NMDA PICs and POCs were readily
distinguishable from the Ca 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 (Figure 5A-B). However, in one
cell we saw the Ca and NMDA PICs come on at separate potentials (as seen by two
negative slope regions in the current response on the upward ramp; not shown).

To eliminate Ca PIC and NMDA PIC interactions, we investigated the action of
NMDA in isolation in 5 rat motoneurons that had the Ca PIC blocked with isradipine
(15 µM), and synaptic input and Na PIC blocked with TTX (Figure 5C). In these
motoneurons, prior to 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 persistent inward current (NMDA PIC, Figure 5D) with the same
asymmetric characteristics followed by a POC, as seen prior to the Ca PIC block. That
is, the NMDA PIC was activated at $-71 \pm 5 \text{ mV}$, again near the pre-drug resting
potential ($-69 \pm 10 \text{ mV}$), and required a negative bias current to prevent activation
(activation current $-0.9 \pm 1.5 \text{ nA}$). This NMDA PIC was on average $-1.7 \pm 0.9 \text{ nA}$
(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 $+0.7 \pm 0.3 \text{ nA}$.

To further investigate 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 (Figure 5E, $N = 5$). Steps over the
resting potential initiated the NMDA PIC with a time to peak current of about 0.5 s.
The net inward current then decreased continuously after this peak, suggesting the
onset of a persistent outward current (POC) that increased with time. This POC
could be seen in isolation when the voltage step was terminated, and then lasted
more than 2 seconds, 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 (Figure 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 re-activated and the whole
process continued, leading to the observed spontaneous oscillations (Figure 6A,
N = 5/5 cells). When additional depolarizing bias current was applied during these
oscillations, the NMDA plateau lasted longer (Figure 6B), thus explaining the longer
bursts of firing seen at depolarized levels without TTX (Figure 3).
Discussion

We demonstrate here that in the adult rodent spinal cord the motoneurons innervating the muscles of the tail are sensitive to bath 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 calcium persistent inward currents (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 (MM, SME and CJH, unpublished results, and Li and Bennett 2003, Murray, 2010), and furthermore, NMDA induced burst persist in a block of Ca PICs and Na PIC. Thus, these bursts are likely mediated by an NMDA current from the NMDA receptors 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 sodium and calcium 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 NMDA receptor, 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 NMDA receptors on adult motoneurons can lead to their direct recruitment, including production of rhythmic bursts of activity. The motoneurons of the sacrocaudal 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 sacrocaudal motoneurons of neonatal rats are able to elicit left-right abductions of the tail, similar to during swimming (Delvolve et al. 2001). Thus our results are consistent with the NMDA-
mediated input to motoneurons occurring during locomotion. A fundamental role of NMDA receptor activation on motoneurons in the adult state during rhythmic activity is supported by the recent study of Enriquez Denton et al. (2012). This study showed that block of NMDA receptors markedly suppressed respiratory oscillations in phrenic motoneurons. Although 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 NMDA receptors blocked were highly likely to be on the motoneurons themselves. There may however also be sensory inputs that activate NMDA receptors on motoneurons. Ia monosynaptic EPSPs are not mediated by NMDA receptors 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 persistent inward Ca and Na currents (Hultborn et al. 2003; Lee and Heckman 2000; Svirskis and Hounsgaard 1998). Thus the voltage-dependent amplification of this sensory input may involve NMDA receptor activation but this possibility has not yet been tested.

While our data clearly demonstrates that NMDA can induce rhythmic alternation in motoneurons, the relative contribution of NMDA oscillations in motoneurons compared to 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, left side) and coordinate the transition to other pool activity (left-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 PICs on motoneurons and explain the tonic activity seen to follow the rhythmic activity in motoneuron pools.

Our finding that NMDA-induced oscillations in motoneurons can sometimes be eliminated by hyperpolarizing a motoneuron does not rule out the involvement of interneurons, because the experimental conditions that we used to make stable intracellular motoneuron recordings do not favor interneuron 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 been seen in motoneurons under hyperpolarized conditions when we reduce or eliminate the glue, though 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 ionotopic inputs
(reviewed by Binder et al. 1996). Perhaps this relatively unexcitable state can be
considered the base state of the motoneuron.

The discovery of Ca PICs (Schwindt and Crill 1977) and their
neuromodulation by the brainstem via axons that release either serotonin or
norepinephrine (Holstege and Kuypers 1987; Hounsgaard et al. 1988; Hounsgaard
and Kiehn 1989) identified a new and highly excitable state of motoneurons
controlled by neuromodulators (second state, neuromodulatory state). Work in
several labs established that PICs are likely to be a standard component of
motoneuron behavior in normal behavior in both animals and humans (reviewed in
norepinephrine also depolarize resting membrane potentials and hyperpolarize
spike thresholds (Binder et al. 1996; Powers and Binder 2001), so that these
brainstem neuromodulatory systems have the capacity to greatly alter the net input-
output gain of motoneurons (Heckman et al. 2008b; Heckman et al. 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, providing
a focused control to oppose the diffuse and widespread effects of the brainstem
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 neuromodulatory inputs originate within the spinal cord (Power
et al. 2010), further contributing to this neuromodulatory state, with for example
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 (third 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 different motor behaviors, depending on the mixture of inputs from brainstem neuromodulatory centers, spinal neuromodulatory, inhibitory 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 remarkable recovery of Ca PIC activity after spinal cord injury, via constitutative activity of receptors for 5HT and NE (Murray et al. 2010; Murray et al. 2011; Rank et al. 2011), but changes in NMDA inputs require further study.

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Disclosures
The authors have nothing to declare.

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Figure Captions

Figure 1. 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 left-side bursts), and increasing with time after application (application 3 mins prior to recording). Eventually tonic synchronous activity also emerged (right of plot).

Figure 2. NMDA-induced bursting in a motoneuron recorded intracellularly

Intracellular recording from a mouse motoneuron. Bath perfusion of NMDA was started at the point indicated by the top arrow, and lasted for the whole duration of the recording. The cell depolarized, and then bursted repeatedly for a minute, followed by tonic firing. The rightmost part of the trace was obtained with a hyperpolarizing bias current of −1.4 nA, and the cell produced repeated short bursts of firing.
Figure 3. Effect of hyperpolarization on the NMDA-induced bursts

Intracellular recording from a mouse motoneuron. Upon 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.

Figure 4. NMDA-induced bursts persist in presence of TTX

This mouse motoneuron exhibited spontaneous bursts of activity in presence of NMDA (second 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.

Figure 5. Currents responsible for the NMDA-induced bursting

A. Ca 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 panel 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 panel 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 panel 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 mV 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.

**Figure 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). 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.
Figure 2
Figure 4
Figure 5
Figure 6

A  Isradipine + TTX + NMDA

B  + depolarizing current

10 mV

10 s