LysRS was obtained by this procedure.
9. For the determination of kinetic parameters, LysRS was added to a final concentration of 5 nM, and the lysine concentration was varied in the range between 0.2 and 5 times the K_M value. Sampling and quantification were as described [K.-W. Hong *et al.*, *EMBO J.* **15**, 1983 (1996)].
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16. For expression in *E. coli*, *M. maripaludis lysS* was subcloned into pET15b (Invitrogen) and then used to transform the strain BL21 (DE3) [W. F. Studier, A. H. Rosenberg, J. J. Dunn, J. W. Dubendorff, *Methods Enzymol.* **85**, 60 (1990)]. This transformation allowed the production of His_6-LysRS, which was subsequently purified by nickel-affinity chromatography (QIAGEN) followed by gel filtration with a Superose 12 column (*8*).
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28. We thank D. Smith and Genome Therapeutics Corporation for access to *M. thermoautotrophicum* genome sequence data before publication; the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University for peptide sequencing and oligonucleotide synthesis; J. Deruère, M. Kitabatake, M. Kumar, M. Prætorius-Ibba, and S. Chaturvedi for advice on cloning strategies and providing materials; and A. Pfeifer and W. B. Whitman for advice and encouragement. Supported by a grant from Bristol-Myers Squibb (D.S.). The sequence of the *M. maripaludis lysS* gene has been deposited in GenBank (accession number AF009824).

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Role of Sensory-Evoked NMDA Plateau Potentials in the Initiation of Locomotion

Gonzalo Viana Di Prisco, Edouard Pearlstein, Richard Robitaille, Réjean Dubuc*

Reticulospinal (RS) neurons constitute the main descending motor system of lampreys. This study reports on natural conditions whereby *N*-methyl-D-aspartate (NMDA)–mediated plateau potentials were elicited and associated with the onset of locomotion. Reticulospinal neurons responded in a linear fashion to mild skin stimulation. With stronger stimuli, large depolarizing plateaus with spiking activity were elicited and were accompanied by swimming movements. Calcium imaging revealed sustained intracellular calcium rise upon sensory stimulation. Blocking NMDA receptors on RS neurons prevented the plateau potentials as well as the associated rise in intracellular calcium. Thus, the activation of NMDA receptors mediates a switch from sensory-reception mode to a motor command mode in RS neurons.

Locomotion can be initiated, guided, and controlled by an array of sensory cues (*1*). Cutaneous inputs elicit bouts of locomotion in intact lampreys (*2*), but the cellular mechanisms by which this occurs are still unknown and are the subject of this study. We used a semi-intact in vitro preparation to characterize the cellular mechanisms responsible for the transition from sensory responses to motor activity, such as swimming (*3*). The advantage of such a preparation is that the sensory inputs are left intact as well as some of the muscles, so that active behavior may be elicited with all the advantages of a standard in vitro preparation. Reticulospinal (RS) neurons constitute the main descending motor system (*4*). They receive sensory inputs from several modalities (*5*–*7*), including cutaneous inputs (*8*), and in turn they make direct synapses with motoneurons and interneurons involved in the segmental generation of locomotion (*9*). Because of their large size and easy access for electrophysiological studies in controlled in vitro conditions, lamprey RS neurons provide an excellent model for investigation of the mechanisms underlying the transformation of sensory inputs into motor commands in vertebrates.

An in vitro brainstem preparation (*10*) was used in which the skin covering the dorsal head region was left attached (Fig. 1A). Mechanical stimulation of the skin elicited postsynaptic potentials (PSPs) in RS neurons (Fig. 1B). The amplitude of the synaptic responses showed a remarkable linear relation with the stimulus strength when mild stimulation was used (Fig. 1B). Under these conditions, RS cells behaved as close followers of sensory inflow, the time course of the excitatory PSPs being perfectly tuned to the variation of the force applied to the skin (inset of Fig. 1D). Because somatosensory inputs to RS neurons involve a di-synaptic pathway, this close relation implies powerful synaptic connections. When stronger stimuli were delivered to the skin, large depolarizing plateaus were elicited, which were accompanied by spiking activity (Fig. 1C); the stimulus-response relation then switched from a linear proportional function to a nonlinear function (Fig. 1D). In a semi-intact preparation (*11*) in which the brainstem and rostral spinal cord were exposed in vitro with the tail left intact to move freely (Fig. 2A), the depolarizing plateaus were accompanied with swimming movements. A long-lasting de-

G. Viana Di Prisco, E. Pearlstein, R. Robitaille, Département de Physiologie, Centre de Recherche en Sciences Neurologiques, Université de Montréal, C.P. 6128, succursale Centre-Ville, Montréal, Québec, Canada, H3C 3J7.
R. Dubuc, Département de Kinanthropologie, Université du Québec à Montréal, Montréal, C.P. 8888, succursale Centre-Ville, Québec, Canada, H3C 3P8 and Département de Physiologie, Centre de Recherche en Sciences Neurologiques, Université de Montréal, C.P. 6128, succursale Centre-Ville, Montréal, Québec, Canada, H3C 3J7.
*To whom correspondence should be addressed. E-mail: dubucr@ere.umontreal.ca

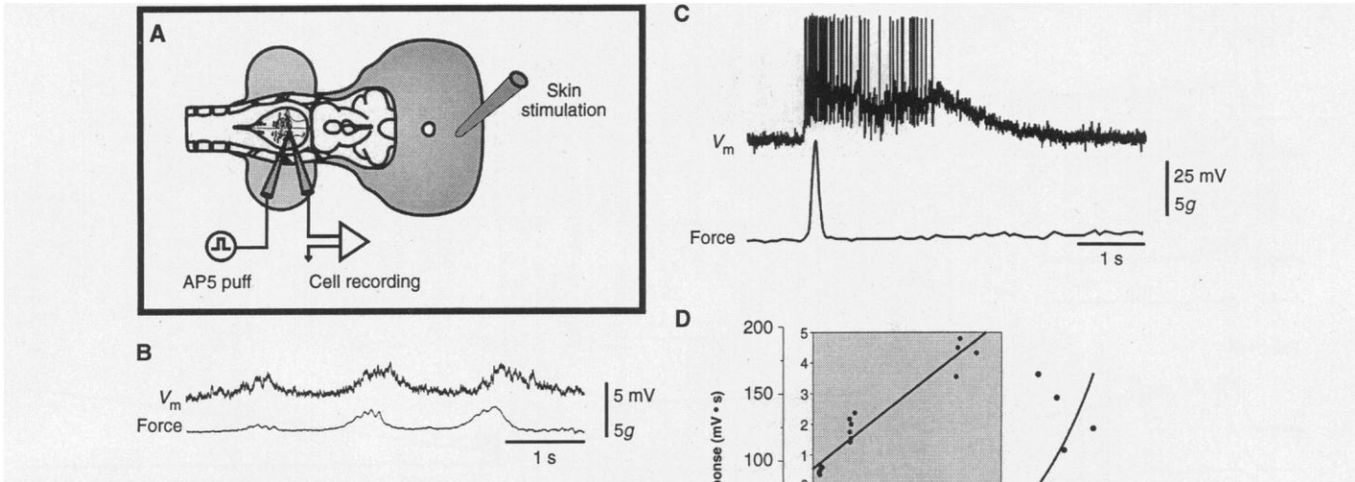

Fig. 1. Responses of RS neurons to mechanical skin stimulation. (**A**) An isolated in vitro lamprey brainstem preparation was used to study RS cell membrane potential response to either direct mechanical stimulation of the skin or electrical pulses delivered to the trigeminal nerve. (**B**) PSPs elicited in RS neurons upon mild skin stimulation. V_m, membrane potential. (**C**) With a stronger mechanical pulse, a longer-lasting depolarization with spiking was induced. (**D**) Stimulus-response curve in one RS neuron upon skin stimulation. The gray area in the main graph is illustrated in the inset. With mild stimuli, the input-response curve showed a linear relation (r = 0.96). With increased stimulation strength, the relation became nonlinear. The stimulus-response relation has been approximated by an exponential growth curve; equation: $y = y_0 + A.e^{(x/t)}$, where y_0 (y offset) = 0, A (amplitude) = 3.00009, and t (time constant) = 0.23453 (r = 0.88).

polarization (plateau potential) occurred, with superimposed spiking activity correlated with the electromyographic (EMG) swimming activity (Fig. 2B). The plateau lasted for the whole duration of locomotor activity. The subthreshold responses elicited by mild skin stimulation were not accompanied by motor responses. The initiation of depolarizing plateau potentials in RS neurons may thus underlie the initiation of locomotor behavior in response to a skin stimulation. As action potentials are generated in the descending axons, the excitability level of the spinal cord networks rises and locomotion is induced.

Because NMDA receptors (NMDARs) are present in the cutaneous pathway to RS neurons (*12*), we tested the possibility that the switch from a linear input-response relation to a nonlinear one may be mediated by properties of the recorded cell such as the activation of NMDARs. The NMDAR blocker 2-amino-3-hydroxy-5-phosphonopentanoate (AP5; 200 μM) was added to the perfusate (Fig. 3A), and its effect on the plateau potentials was tested. Under this condition, the long-lasting depolarizing plateaus were abolished (n = 6). However, because AP5 may also have affected transmission at the synaptic relay, it was applied locally by pressure-ejecting it on the surface of the recorded RS cell. The relay cells were located in the alar plate more than 800 μm away and thus were well beyond reach of the injection. This local application abolished the long-lasting depolarizing plateaus (Fig. 3B), which indicates that NMDARs located on the postsynaptic membrane of RS neurons are involved in mediating the plateaus. Moreover, the nonlinear relation in the input-response curve disappeared after AP5, as plateau potentials could not be elicited under these conditions (Fig. 3C) (*13*).

The activation of NMDARs leads to Ca^{2+} entry into the cell. To further elucidate the role of NMDARs in RS neurons, we used calcium imaging (*14*). We studied the pattern of activation of groups of RS neurons in different brainstem reticular nuclei immediately after stimulation of trigeminal afferents (either by direct electrical stimulation of the nerve or by mechanical stimulation of the skin covering the snout) (Fig. 4A). We found that with low-intensity stimulation, transient Ca^{2+} responses occurred in populations of RS neurons including the Mauthner cells (Fig. 4B). With stronger intensities, a nonlinear sustained Ca^{2+} plateau response appeared, which lasted for several seconds or minutes. These Ca^{2+} plateaus were accompanied by depolarizing plateau membrane potentials, as confirmed with simultaneous optical imaging and intracellular recordings (Fig. 4C). The application of AP5 practically abolished not only the depolarizing plateaus but also the increase in intracellular Ca^{2+} in the same neurons (n = 3, Fig. 4D), which indicates that NMDARs are also involved in the Ca^{2+} response.

The present results indicate that identified RS neurons in the lamprey have an inherent nonlinearity that allows them to generate long-lasting, NMDA-dependent depolarizing plateaus accompanied by Ca^{2+} entry into the cell. The occurrence of these

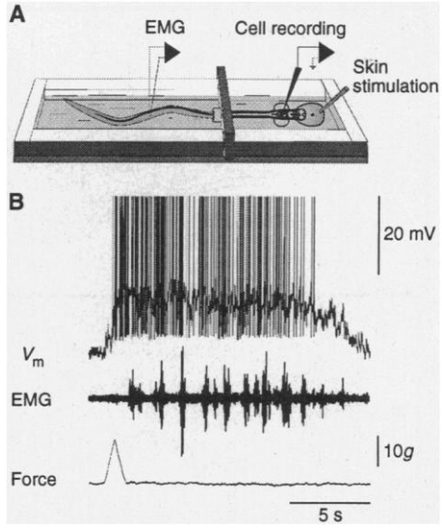

Fig. 2. Plateau potentials and the onset of locomotion. (**A**) Schematics of a semi-intact preparation, where the tail is left intact and can freely swim. EMG activity was recorded from the midbody region on the side contralateral to the intracellular recording. (**B**) Mechanical stimulation of the skin covering the head region elicited a plateau of depolarization in an RS cell. After the onset of the plateau, locomotor movements of the tail were elicited. Note the bursts of discharge in the EMG signal.

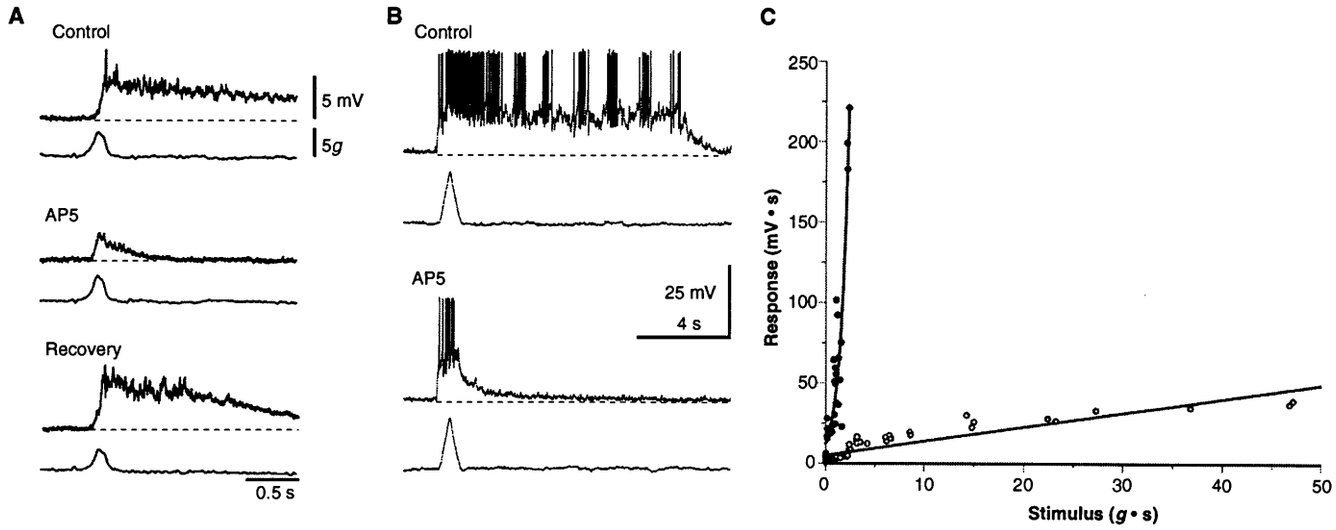

Fig. 3. The effect of the NMDA receptor antagonist AP5 on plateau potentials. (**A**) Plateau potentials in RS cells evoked by skin stimulation were dramatically reduced when AP5 (200 μM) was added to the Ringer's solution. (**B**) Local application of AP5 (1 mM) was equally effective in reducing plateau potentials. (**C**) Stimulus-response curve in one RS neuron before AP5 (solid circles; exponential growth curve fitting $y = y_0 + A.e^{(x/t)}$, where $y_0 = 0$, $A = 12.23112$, and $t = 0.80898$; $r = 0.92$) and after AP5 (open circles; curve is a linear regression fit; $r = 0.91$).

plateaus makes the neuron switch from a sensory-reception mode to a motor command mode that activates the spinal locomotor networks. NMDARs are known to play an important role in both sensory and motor systems (*15*). In particular, there is ample evidence that excitatory amino acid transmission, especially that involving NMDARs, is involved in the generation of locomotion in vertebrates such as lampreys, frogs, rats, and cats (*16*). This study establishes a new role of NMDARs in integrated behavior related to their voltage-dependent properties and linked to the brain-stem initiation of locomotion.

The initiation of locomotion in the lamprey is likely to require the activation of several RS cells. We report here that RS cells behave in a similar fashion to invertebrate "command neurons" that when stimulated elicit coordinated movements (*17*). Indeed, dorsal ramp interneurons that trigger swimming in the marine mollusk *Tritonia diomedea* display similar plateau potential properties (*18*). The depolarization plateaus may be a general feature by which rapid motor commands are elicited in response to sensory cues in several animal species, including humans (*19*).

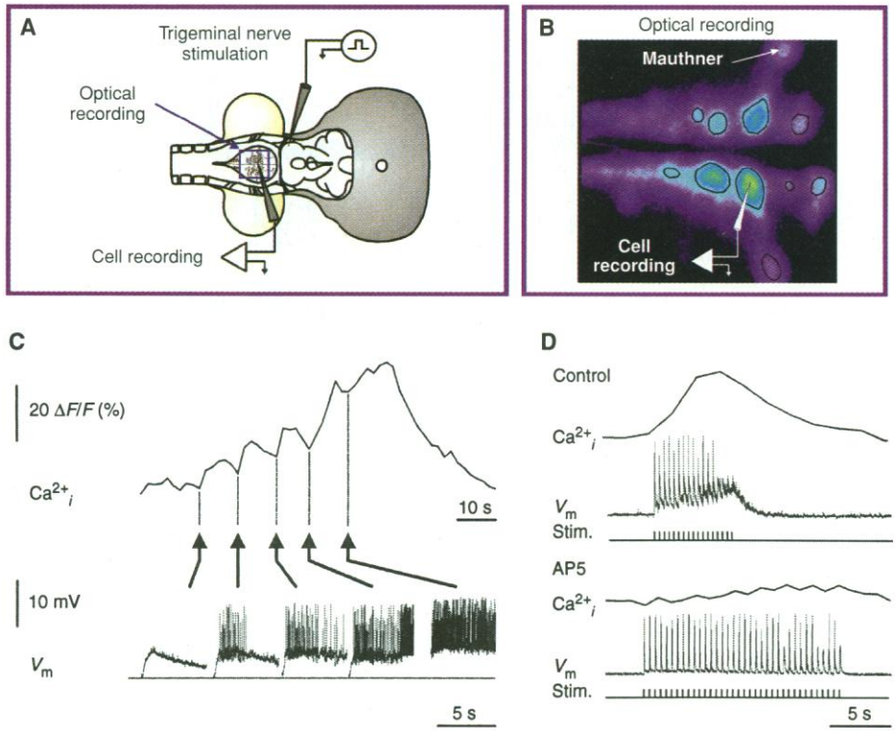

Fig. 4. Ca^{2+} responses in RS neurons. (**A**) Schematics of the experimental setup for Ca^{2+} imaging of RS cells. (**B**) RS neurons in the middle rhombencephalic reticular nuclei displayed an increase in fluorescence when the trigeminal nerve or the skin covering the head region was stimulated. (**C**) Calcium response (Ca^{2+}_i, top trace) of an RS cell soma that was simultaneously recorded with a sharp microelectrode (V_m, lower trace). The windup of depolarization and spiking induced by repeated stimuli (indicated by arrows) coincided with an increase in fluorescence. (**D**) An example from a different cell, showing that both the depolarizing plateau (V_m) and the calcium response (Ca^{2+}_i) were depressed by the addition of AP5. The calibrations for V_m and Ca^{2+}_i are the same in (C) and (D).

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11. The semi-intact preparation (n = 11) was similar, but the caudal two-thirds of the body was left freely moving behind the dissected brainstem and rostral spinal cord. Teflon-coated stainless steel microwires (50 μm in diameter) were inserted into the muscle for EMG recording.
12. Trigeminal inputs to RS neurons in lampreys are mediated by excitatory and inhibitory amino acids (*6*, *20*).
13. Consistent with this observation, we recently recorded NMDA-induced plateau potentials, which are resistant to tetrodotoxin, in RS neurons.
14. Reticulospinal neurons were retrogradely labeled in vitro for 24 to 48 hours by placement of Calcium Green–Dextran (10,000 MW; Molecular Probes) on the rostral end of the sectioned spinal cord at the 1–2 segment level. Labeled cells were imaged on a Nikon epifluorescent microscope and recorded with an intensified charge-coupled device camera at a rate of one to two images per second. Calcium responses are expressed as relative changes in fluorescence ($\Delta F/F$ %). M. J. O'Donovan, S. Ho, G. Sholomenko, W. Yee, *J. Neurosci. Methods* **46**, 91 (1993); A. D. McClellan, D. McPherson, M. J. O'Donovan, *Brain Res.* **663**, 61 (1994); D. A. Nelson and L. C. Katz, *Neuron* **15**, 23 (1995); D. M. O'Malley, Y.-H. Kao, J. R. Fetcho, *Neuron* **17**, 1145 (1996).
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Structural Plasticity in a Remodeled Protein-Protein Interface

Shane Atwell, Mark Ultsch, Abraham M. De Vos, James A. Wells*

Remodeling of the interface between human growth hormone (hGH) and the extracellular domain of its receptor was studied by deleting a critical tryptophan residue (at position 104) in the receptor, creating a large cavity, and selecting a pentamutant of hGH by phage display that fills the cavity and largely restores binding affinity. A 2.1 Å resolution x-ray structure of the mutant complex showed that the receptor cavity was filled by selected hydrophobic mutations of hGH. Large structural rearrangements occurred in the interface at sites that were distant from the mutations. Such plasticity may be a means for protein-protein interfaces to adapt to mutations as they coevolve.

Protein-protein interfaces are usually large and elaborate, consisting of 10 to 40 contact side chains, each of which interdigitates with several others across the interface (*1*). The contact side chains are often presented from discontinuous segments of each polypeptide chain. Given the complexities of these interactions, we wondered how a functionally disruptive mutation on one side of the interface could be complemented by mutations in its binding partner. This is a challenge that nature faces as protein complexes coevolve.

We studied this problem by phage display using the high-affinity interface between hGH and the extracellular domain of its receptor (hGHbp), members of a cytokine-receptor superfamily (*2*). The hormone and receptor initially form a tight 1:1 complex (dissociation constant K_d = 0.3 nM), and the x-ray structure of this complex is known to high resolution (*3*). There are about 30 contact side chains on each side of this interface, but alanine-scanning mutagenesis has shown that only a small set of primarily hydrophobic contacts at the center of the interface dominate affinity (*4*, *5*). This energetic "hot spot" on the recep-

S. Atwell and J. A. Wells, Department of Protein Engineering, Genentech, Incorporated, 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA, and Department of Biophysics and Biochemistry, University of California, San Francisco, CA 94143, USA.
M. Ultsch and A. M. De Vos, Department of Protein Engineering, Genentech, Incorporated, 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA.
*To whom correspondence should be addressed.