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Life Sciences 72 (2002) 389–396
Life Sciences
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# Corticospinal transmission to motoneurons in cervical spinal cord slices from adult rats

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**Abstract**

Cervical spinal cord slices were prepared from adult rats. Intracellular recordings from motoneurons revealed that electrical stimulation of the ventralmost part of the dorsal funiculus (which contains primarily descending corticospinal axons) elicited EPSPs in 75% of the neurons. The latencies of these EPSPs tended to be shorter than those elicited by dorsal horn gray matter stimulation. Pairs of subthreshold dorsal funiculus stimuli were able to elicit action potentials in motoneurons. These data are consistent with previous morphological and electrophysiological studies indicating that cervical motoneurons receive both mono-and polysynaptic corticospinal inputs. In addition, motoneurons were markedly depolarized by iontophoretic application of AMPA or KA (7 out of 7 neurons), but only weakly depolarized by NMDA (1 out of 6 neurons). CNQX (but not AP-5) blocked EPSPs elicited by dorsal funiculus stimulation. Thus, corticospinal transmission to motoneurons is mediated primarily by non-NMDA glutamate receptors.


*Keywords:* Motoneuron; Cervical spinal cord; Slice; Adult rat

## Introduction

Corticospinal neurons exert both direct and indirect control over spinal motoneurons [5,8]. Direct corticospinal effects on spinal motoneurons are presumed to be mediated by excitatory amino acids [9] although the nature of the changes in conductance resulting from monosynaptic corticospinal transmission to motoneurons has not been evaluated pharmacologically.

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0024-3205/02/$ - see front matter © 2002 Published by Elsevier Science Inc.
PII: S0024-3205(02)02279-8

Glutamatergic transmission to spinal motoneurons has been primarily investigated using in vitro preparations from neonatal or fetal animals [1,4,11,13,15,16]. However, spinal glutamatergic transmission changes throughout postnatal development. For example, NMDA receptors are lost from all areas of the spinal gray matter except the substantia gelatinosa during the second and third postnatal weeks [13]. In addition, the NR1 subunit of the NMDA receptor is expressed at higher levels in the somata and dendrites of neonatal motoneurons than in those of adult animals [18]. During this early postnatal period, both NMDA and non-NMDA receptors are transiently overexpressed in the spinal cord, with non-NMDA receptor-mediated excitatory transmission gradually becoming more dominant with increasing age [10,17]. These age-related changes in the expression of NMDA receptors have also been observed in higher brain regions, such as in Meynert neurons [3]. Thus, evaluation of glutamatergic transmission using in vitro preparations from neonatal and young animals is unlikely to provide an accurate description of glutamatergic transmission to spinal motoneurons in the adult.

Methods for studying motoneurons from adult in vitro spinal cord preparations comparable to those from neonatal preparations have only recently been developed. Jiang et al. [12] and Carlin et al. [6] studied motoneurons in thin spinal cord slices from functionally mature, although still young, mice. However, the question of corticospinal transmission in adults remains unresolved. Recently, we have developed a method enabling electrophysiological and anatomical study of motoneurons in cervical spinal cord slices from rats up to 3 months old [10]. In the present study, we employed this method to study glutamatergic transmission to spinal motoneurons in response to stimulation of corticospinal axons in the ventralmost part of the dorsal funiculus.

## Methods

Experiments were performed on transverse slices from the C4–C8 spinal cord of 25 mature, male Wistar rats (weighing 150–180 grams, approximately 2.5 months old). The methods have been described previously [9] and are summarized here. Animals were deeply anesthetized with ether and perfused transcardially with a cold, low-sodium, oxygenated (95% $O_2$/5% $CO_2$) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 212.5 sucrose, 3.5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.2 $KH_2PO_4$, and 10 glucose (cf. [2]). The cervical spinal cord was exposed by dorsal laminectomy while the spinal cord was continuously bathed with cold, low-sodium ACSF. Spinal roots were cut, and a piece of spinal cord comprising the C1–Th3 segments was laid in a slot in a chilled agar block. The cord was covered by a second chilled agar block and then this entire assembly was glued to the stage of a vibrating microtome (Nomiyama Rika, Japan) and bathed in the cold, low-sodium ACSF. Thick (450 μm) transverse slices were cut (i.e., perpendicular to the longitudinal axis of the spinal cord). After incubating the slices in the low-sodium ACSF for at least one hour at 34 °C, they were transferred to normal ACSF of the following composition (in mM): 125 NaCl, 3.5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.2 $KH_2PO_4$, and 10 glucose, where they were incubated for at least another 30 minutes. Individual slices were then transferred to a recording chamber and superfused with normal ACSF at 3 mL/min at 34 °C.

Spinal neurons were impaled with glass micropipettes filled with 3 M $K^+$ acetate. In some cases, electrodes also contained Lucifer Yellow CH (Sigma, 10% in distilled water) which was injected into the neurons for subsequent anatomical verification using 2 nA negative current pulses of 250 msec duration,

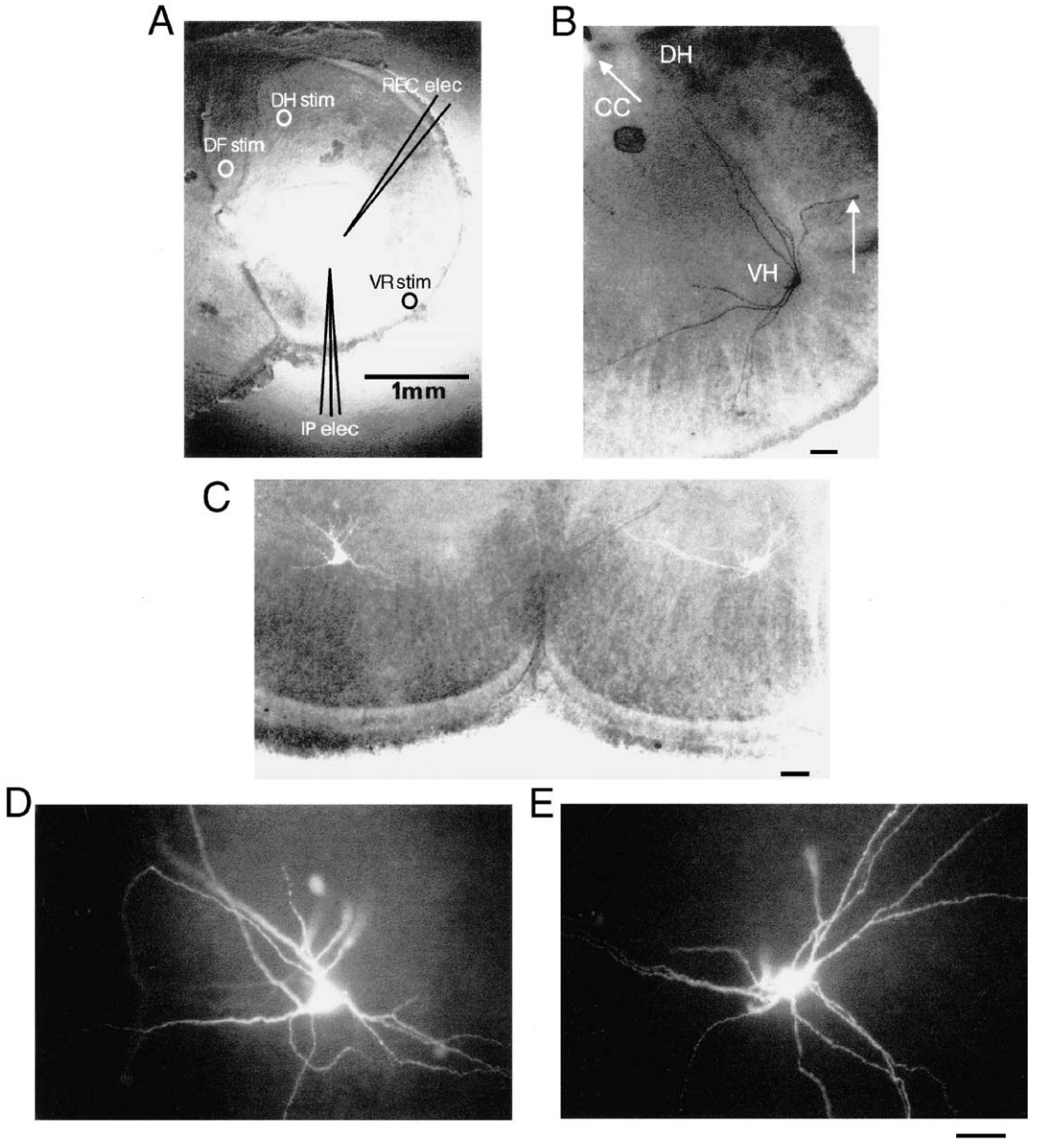


Fig. 1. (A) Micrographs of a transverse section of the rat cervical spinal cord illustrating the typical positions of the recording electrode (REC elec), the iontophoretic electrode (IP elec), and the three positions for the stimulation electrodes: in the ventral root entry zone (black circle), in the ventral part of the dorsal column (white circle), and in the dorsal horn (white circle). (B) A motoneuron filled with Lucifer Yellow, illustrating its cell body in the ventral horn, its dendrites projecting into both the ventral and dorsal gray matter and an axon (longer arrow) projecting into the ventral root. CC (arrow), central canal; DH, dorsal horn; VH, ventral horn. (C) Symmetrically arranged motoneurons in the same plane. (D) Enlargement of left-hand neuron shown in C. (E) Enlargement of right-hand neuron shown in C. Scale bars are 50 μm (both D and E use the scale bar under E).

applied at 2 Hz for 1.5–2.0 min. Intracellular recordings were made and responses to applied agonists and electrical stimulation were recorded. AMPA, NMDA, and KA (10 mM in 0.15 M NaCl, pH 7.5) were iontophoretically applied through a multi-barreled micropipette placed independently of the recording electrode. Electrical stimulation was delivered to different sites in the slice using concentric electrodes with a 30-μm diameter internal electrode (cathode) that protruded 300 μm from the tip of the outer electrode. Fig. 1 illustrates the positions of the intracellular recording electrode, the iontophoretic multi-barreled pipette, and the stimulating electrodes in the ventral root, dorsal horn grey matter, and dorsal funiculus. Input resistance was determined from the voltage response to intracellularly injected hyperpolarizing current pulses.

## Results

Intracellular recordings were made from 67 neurons with cell bodies in lamina IX. All of these cells were considered to be motoneurons on the basis of their physiological and anatomical properties. Stimulation of the ipsilateral ventral root (VR stim in Fig. 1) elicited single antidromic action potentials with very short latencies (less than 0.2 ms) in 85% of these neurons (illustrated in Fig. 2A). The average motoneuron resting potential and input resistance of neurons in which antidromic action potentials could be evoked were $-65 \pm 5$ mV and $23.4 \pm 2.5$ MΩ, respectively (mean ± S.D., n = 11). Electrical stimulation of ventral roots at intensities below the action potential threshold usually elicited only IPSPs (presumably due to activation of Renshaw cells). The remaining 15% of neurons in which only IPSPs could be elicited had values of resting membrane potentials, action potential amplitudes (in response to somatic injection of current), and input resistances comparable to those observed in neurons with antidromic action potentials.

Regardless of the ability to elicit action potentials antidromically, stimulation in the dorsal horn gray matter (e.g., DH stim in Fig. 1) always elicited action potentials with very short latencies (less than 0.2 ms) (Fig. 2B). These action potentials probably resulted from direct stimulation of dendrites extending into this region, since they were not blocked by exposure to ACSF with low $Ca^{+2}$ (0.1 mM) and high $Mg^{+2}$ (4.3 mM), which would be expected to block synaptically evoked responses. In addition, dorsal horn stimulation at intensities below the threshold for inducing action potentials elicited EPSPs with much longer latencies in all of these neurons. The average latency from the stimulus artifact to the onset of the EPSP was $2.1 \pm 0.5$ ms (mean ± S.D, n = 6). These long-latency EPSPs were blocked by exposure to solutions of low $Ca^{+2}$ and high $Mg^{+2}$.

Stimulation of the ventral portion of the ipsilateral dorsal funiculus (DF stim in Fig. 1), which primarily contains axons of the main corticospinal tract, evoked EPSPs in 75% of the motoneurons (illustrated by the response to the first stimulus in each of the three traces of Fig. 2C). The average latency of these EPSPs was $1.0 \pm 0.3$ ms (mean ± S.D., n = 9, Fig. 2D). Action potentials could be elicited by a single stimulus of sufficient intensity (Fig. 2D) or by application of pairs of subthreshold stimuli. Fig. 2C shows that action potentials induced by such paired pulse stimulation, with different inter-pulse intervals, occur with the same delay.

Pyramidal neurons that comprise the corticospinal tract have been suggested to use glutamate or aspartate as their neurotransmitters [9]. We assessed the role of excitatory amino acid neurotransmitters in transmission from these descending corticospinal fibers to motoneurons by bath application of glutamate receptor antagonists in 7 cells. Typical results are shown in Fig. 2E. A 5-minute exposure to

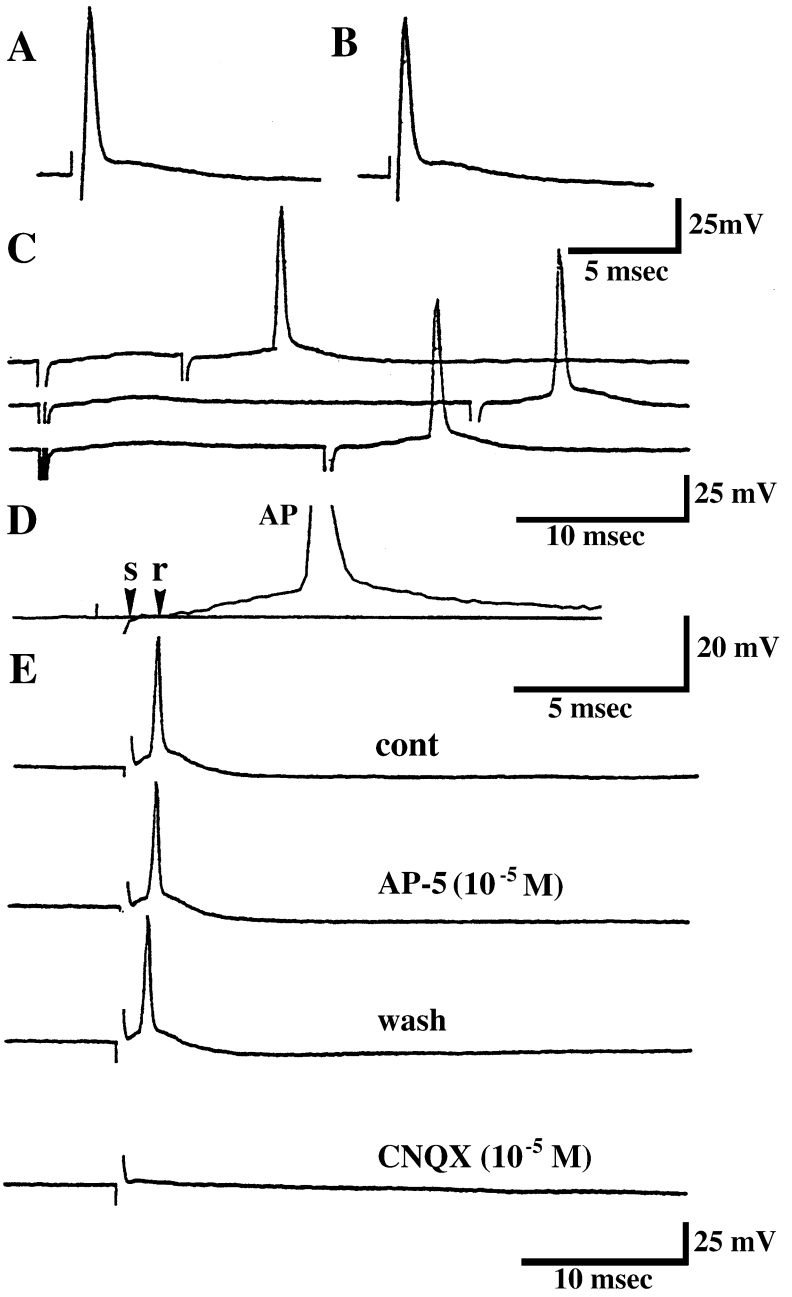


Fig. 2. Typical responses recorded from motoneurons. (A) Antidromic response to stimulation of the ventral root (VR stim in Fig. 1). (B) Response evoked by direct stimulation of the motoneuron's dendritic tree which extended into the dorsal horn (DH stim in Fig. 1) (C) Responses to paired pulse stimulation of descending corticospinal fibers in the ventral dorsal funiculus (DF stim in Fig. 1). (D) Response evoked by descending fiber stimulation, displayed using an expanded time scale. s, stimulus artifact; r, EPSP onset. (E) Effect of bath perfusion of AP-5 ($10^{-5}$ M) and CNQX ($10^{-5}$ M) on the response of a motoneuron to stimulation of the ventral dorsal funiculus (DF stim in Fig. 1).

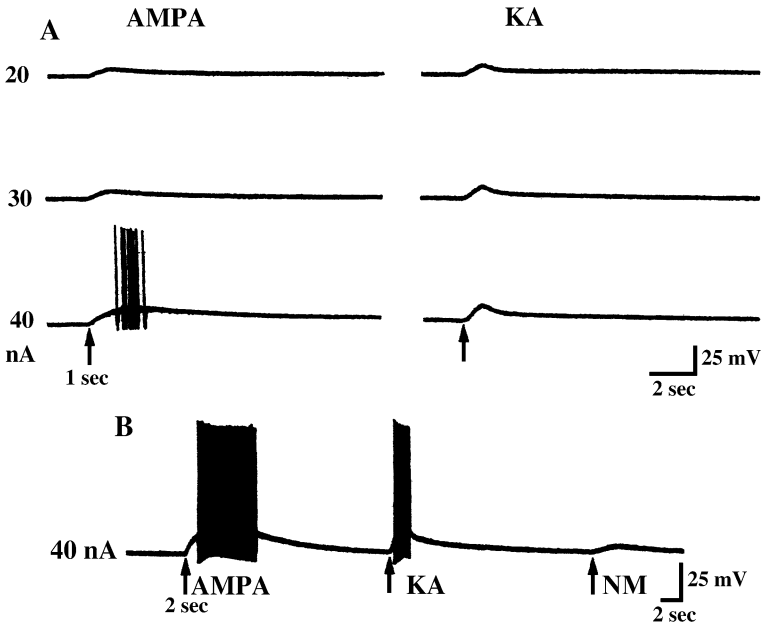


Fig. 3. (A) Typical motoneuron response to iontophoretic application of AMPA (left) and KA (right), with the magnitude of the depolarization being dependent on the ejection current applied to the iontophoretic electrode. (B) Prolonged iontophoretic application of AMPA and KA depolarizes the motoneuron and elicits repetitive action potential firing. The response to prolonged application of NMDA in the same motoneuron is only small. Arrows indicate the onset of iontophoretic application.

$10^{-5}$ M AP-5, an NMDA receptor blocker, had no effect on the evoked synaptic responses. In contrast, application of $10^{-5}$ M CNQX markedly reduced the EPSP by about 95%. In 4 of these 7 neurons, raising the concentration of CNQX to $5 \times 10^{-5}$ M had no further effect.

To further evaluate the pharmacological sensitivity of corticospinal tract-induced excitation of motoneurons, AMPA, KA, and NMDA were iontophoretically applied to antidromically identified motoneurons (n = 7). Fig. 3 shows typical results for data obtained from a single motoneuron (resting potential = − 71 mV, antidromic action potential amplitude = 93 mV, and membrane resistance = 26.4 MΩ). Only 1 of the 7 cervical motoneurons tested was depolarized by NMDA at iontophoretic current levels that were effective with the other agonists. Long-duration and high-current iontophoretic application of NMDA consistently depolarized motoneurons, but only modestly (Fig. 3B). In addition, NMDA-elicited responses were still not observed when the membrane potential was depolarized or when NMDA was applied in a $Mg^{+2}$-free ACSF solution containing 10 mM glycine. Fig. 3A shows examples of the concentration dependence of responses to AMPA and KA. In contrast to NMDA, all motoneurons showed large depolarizing responses to application of these agonists, even when using iontophoretic current pulses of only moderate amplitude.

## Discussion

Modification of standard methods for preparing CNS tissue slices enabled us to successfully maintain viable adult spinal ventral and dorsal horn neurons and with neuronal connections comparable to those

observed in vivo. As shown in Fig. 1, motoneuron dendrites extended into the ipsilateral dorsal horn. Stimulation of the ipsilateral ventral root elicited antidromic action potentials in these motoneurons, while stimulation of the dorsal horn and the ipsilateral dorsal funiculus elicited synaptic responses. In the small proportion of neurons (15%) in which antidromic action potentials could not be elicited, ventral root stimulation still evoked IPSPs. We presume that these are also motoneurons whose axons have been cut off and that receive inhibitory synaptic input via Renshaw cells, activated by action potentials evoked in adjacent motoneurons.

Previous anatomical studies have demonstrated the existence of monosynaptic contacts of corticospinal tract fibers onto spinal motoneurons [5,7,14]. In addition, Elger et al. [8] have reported both mono-and polysynaptic responses of cervical motoneurons to epicortical stimulation in rats in vivo. The data presented here are consistent with these findings. The distance between the ipsilateral dorsal funiculus and lamina IX in these slices was approximately 2 mm, while the average latency of the motoneuron EPSP in response to dorsal funiculus stimulation was 1 msec. That this latency is somewhat longer than that observed in vivo may be accounted for by the lower temperature at which it was recorded in vitro. This latency tended to be shorter than that elicited by stimulation of the dorsal horn at a similar distance from the recorded motoneuron. Both the relatively short EPSP latency after dorsal funiculus stimulation and the demonstration of paired pulse EPSP facilitation are consistent with a functional connection that is at least partly monosynaptic between corticospinal fibers and cervical motoneurons.

Pharmacological identification of the neurotransmitter(s) involved in corticospinal-evoked EPSPs in motoneurons was investigated using bath application of the glutamate receptor antagonists AP-5 and CNQX and by using iontophoretic application of the agonists KA, AMPA, and NMDA. The results clearly support the hypothesis that excitatory synaptic transmission from corticospinal fibers to spinal neurons is predominantly glutamatergic, and is mediated via non-NMDA glutamate receptors. The residual responses that could not be eliminated by a high concentration of CNQX presumably reflects a small non-glutamatergic component, perhaps due to activation of other transmitter systems in the adjacent dorsal horn or white matter.

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