In Vivo Recordings of Bulbospinal Excitation in Adult Mouse Forelimb Motoneurons

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Alstermark, Bror and Jun Ogawa. In vivo recordings of bulbospinal excitation in adult mouse forelimb motoneurons. J Neurophysiol 92: 1958–1962, 2004. First published April 14, 2004; 10.1152/jn.00092.2004. Here we report on pyramidal and reticulospinal excitation in forelimb motoneurons in the adult mouse using intracellular recordings in vivo. The results have been obtained in BALB/C mice, which were anesthetized with midazolam fentanyl/fluanison. In contrast to the rat, only weak and infrequent pyramidal excitation could be evoked with a minimal trisynaptic linkage. Dipsynaptic reticulospinal excitation could also be evoked, as well as monosynaptic reticulospinal excitation from the medial longitudinal fasciculus. The results suggest that reticulospinal pathway in the mouse is important in voluntary motor control of the forelimbs and that the role of the corticospinal tract might be different in mouse compared with rat. Our study provides an opening for studying the effect of genetic manipulation on specified descending systems in the mouse in vivo.

INTRODUCTION

The mouse has become a particularly important model for physiological investigations after the mouse genome project (Mouse Genome Sequencing Consortium 2002), and there is a dramatic increase in the use of transgenic animals for exploring the functional role of specific genes. This is also true for the brain, where much work is devoted to developmental control, diseases, and in vitro studies of neuronal properties in neonates. A major difficulty for neurophysiological investigations has been to perform intracellular recordings from motoneurons in vivo in the adult mouse. Since the work by Kuno and colleagues (Huizar et al. 1975; Kuno 1976) on the electrophysiological properties of spinal motoneurons in normal and dystrophic mice, no publications using intracellular recordings in vivo. The results have been obtained in BALB/C mice, which were anesthetized with midazolam fentanyl/fluanison. In contrast to the rat, only weak and infrequent pyramidal excitation could be evoked with a minimal trisynaptic linkage. Dipsynaptic reticulospinal excitation could also be evoked, as well as monosynaptic reticulospinal excitation from the medial longitudinal fasciculus. The results suggest that reticulospinal pathway in the mouse is important in voluntary motor control of the forelimbs and that the role of the corticospinal tract might be different in mouse compared with rat. Our study provides an opening for studying the effect of genetic manipulation on specified descending systems in the mouse in vivo.

METHODS

Preparation

The results were obtained from 15 mice (5 females and 10 males; BALB/C, Mollegaard), with body weights of 24–30 g and age of 2–4 mo. The animals were anesthetized with a mixture (initial dose, 0.15 ml/30 g ip supplemented with doses of 0.02 ml, maximal dose 0.25 ml) of midazolam (2.5 mg/ml) and fentanyl/fluanison (5.1 mg/ml). Atropin (total dose, 0.5 mg) and decadrone (total dose, 0.4 mg) were always given (subcutaneously) just after anesthesia. Ephedrine was given in doses of 0.1 mg when PCO₂ decreased below 1% for >5 min (intrapertoneal initial dose of 0.2 ml was given 30 min after starting anesthesia and repeated every 30 min). Tracheotomy, pneumothorax, and artificial respiration (rate, 60/min; volume, 50-ml flow O₂) were always performed; the animals were immobilized with gallamine triethiodide (Flaxedile; 2 mg/h ip). The respiratory pump was built by Staffan Berg (University of Göteborg) and consisted of a rotatory disk with a slit. The pneumothorax was carefully made using fine scissors. A 5-mm-long hole was made parallel to the bones. This hole provided an effective elimination of pressure variations and also prevented the lungs from drying. Rectal temperature was maintained at 36–38°C, and the heart rate (~500/min) and expiratory CO₂ were monitored continuously and kept within a physiological range. Large variations in CO₂ was observed (initial value, ~6.0% before artificial respiration was started, which decreased to 3.0% after artificial respiration). The pCO₂-meter (Datex type CD-200-23-00, Instrumentarium Corp., Helsinki, Finland) was adapted for the small expiratory volume in the mouse. The animal was mounted in a head holder built by Lennart Näslund (Umeå University) for stereotaxic placement of brain electrodes. The body was stretched by pulling the hindlimbs with strings attached to the feet. The forelimbs were stretched slightly downward by strings attached to the paws.

A laminectomy was performed that exposed spinal segments C₂–C₇. The deep radial (DR) and superficial radial (SR) nerves were dissected and mounted on bipolar stimulating electrodes in a paraffin pool or stimulated with inserted needle electrodes through the skin. The DR and SR nerves were used for guidance to find the lateral motor nuclei and to check the physiological integrity of the spinal cord after the lesion. The dorsal column was transected at the C₂ level in three experiments to interrupt the corticospinal tract (Fig. 3D). A posterior craniotomy was performed, exposing the cerebellum and the caudal brain stem.

Stimulation and recording

Corticofugal fibers were stimulated in the ipsi- and contralateral pyramids at 0.3–0.5 mm lateral to the midline, 1.5 mm rostral to the obex level with a rostral angle of 30° using tungsten electrodes (100-kΩ impedance, uninsulated tips of 10 μm diam). The threshold using 1-μs pulses was always <10 μA and usually between 5 and 10 μA. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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10 μA for the corticospinal volley. A train of two to four stimuli given at 300 Hz and 80–100 μA was usually used. Recording of the descending volley was made from the surface of the dorsal column (DC), in the middle part of the C2–C7 segments using a silver ball electrode. Intracellular recording was made with borosilicate glass micro-electrode, tip diameter around 0.5–1 μm and impedance of 10–30 MΩ and filled with 2 M potassium citrate (pH 7.4) with a minimal membrane potential of ~40 mV. Stable intracellular recording could usually be maintained between 5 and 15 min. The condition of the animal was good for intracellular recording between 3 and 5 h. All signals were digitized, stored on hard disk (Digidata 1200, Axon Instruments), and analyzed off-line in Clampfit (Axon Instruments).

Due to the short distances in the mouse, current spread is a major problem when stimulating electrically. This was checked by testing occlusion (due to co-activation) and summation (independent activation) of the descending corticospinal volleys evoked from the ipsi- and contralateral pyramids (see RESULTS and Fig. 2E). We found that 100 μA causes effective spread within a radius of ~300 μm. The stimulating electrodes were positioned most ventrally in the pyramids. We regularly used current strengths between 80 and 100 μA to minimize activation of fibers located outside the pyramids.

To calculate the conduction velocity of the descending volley, measurements of the distance were made in situ. The location of stimulating and recording electrodes was verified, and the extent of the lesion was reconstructed from sections stained with neutral red.

This investigation was approved by the ethical committee of Umeå University.

RESULTS

Corticospinal field potential and volley

The termination of the corticospinal fibers in C7 was assessed by recording the extracellular field potentials evoked by a single pyramidal stimulation (contralateral side) as shown in Fig. 1, A and B. The recording tracks were spread from 15° laterally to 20° medially and covered a major part of the gray matter (Fig. 1A). Sampling was made at 200-μm intervals from the surface (200–800 μm). As shown in Fig. 1B, a small negative monosynaptic (latency, 0.5 ms) field potential appeared at 200-μm depth from the surface and became maximal in amplitude at depths of 400–600 μm in track 20° medially. In tracks 10° medially and 0°, 10°-15° laterally, the negative field potential appeared gradually more dorsally as outlined by the interrupted line.

Thus at 15° laterally, the negative field potential could only be recorded at depth 200 μm, which corresponds to the dorsal horn. The area with negative field potentials is indicated by the interrupted line in Fig. 1A and covered the dorsal horn, lamina V, lamina VI, and the medial part of lamina VII. Small positive field potentials were recorded in the lateral part and ventrally in lamina VII and in the motor nuclei (lamina IX). These results suggest that the major termination of the corticospinal fibers in the mouse is in the dorsal horn and medially in the intermediate layers, but not laterally in the intermediate layers and not in the motor nuclei. This pattern of termination is supported by labeling of corticospinal fibers in the adult mouse (T. Isa, personal communication). With respect to laminae VI-VII, the termination in the mouse differs compared with the rat, where it has been found also laterally and is larger in amplitude (Alstermark et al. 2004).

Figure 1C shows a recording from the cord dorsum of the descending corticospinal (top), and for comparison, the medial longitudinal fasciculus (MLF; bottom) volleys (onset indicated

FIG. 1. Corticospinal field potential and descending volleys. A: transverse section from mid-C7 showing 5 electrode tracks at different angles used for the extracellular field recordings. Interrupted line delineates region of negative pyramidal field potentials. B: extracellular field potentials recorded at intervals of 200 μm from depth 200–800 μm from the surface. Horizontal baseline has been added. Negative field pyramidal field potentials are circled by the interrupted black line. C: recordings made with a silver ball electrode on the dorsal column of the volleys and cord dorsum potentials in mid-C2–C3 segments. Corticospinal fibers were stimulated electrically in the contralateral pyramid at a strength of 100 μA. Black arrows show onset of the negative component of the corticospinal and medial longitudinal fasciculus (MLF) volleys (note the different time scales for the corticospinal and MLF volleys). D: latency of descending volley as function of conduction distance from the pyramidal (filled circles and solid line) and MLF (open circles and dotted line) stimulation to segmental recording site. Arrival time in mid-C2 border is shown by the gray line for the MLF volley and by the black line for the corticospinal volley.
by filled arrows) evoked by electrical stimulation (100 μA). Recording was made with a silver ball electrode positioned on the dorsal column in the middle of each segment from C₂ to C₇. For comparison, the onset of the volley has been adjusted to the horizontal line. In case of the corticospinal volley, it can be seen that the amplitude in C₃–C₄ was virtually identical and decreased gradually in C₅–C₇, which suggests that there was a major termination in the caudal cervical segments similar to the rat (Alstermark et al. 2004). In contrast to the rat (Alstermark et al. 2004), the volley remained rather synchronized in the caudal segments C₅–C₇, which indicates that the corticospinal fibers are fairly homogenous with respect to size. The conduction velocity was 8.7 ± 2.4 (SD) m/s (n = 10); similar velocity was recently found by Tanaka et al. (2004), which is similar to the slow component in the rat (Alstermark et al. 2004). As shown in Fig. 1C (bottom) stimulation in the MLF evoked a descending volley (shown with faster time base), which remained virtually unchanged in amplitude from C₂ to C₇, and had a conduction velocity of ~50 m/s (as recently found by Tanaka et al. 2004).

Figure 1D shows the latency of the corticospinal (Pyr; solid line and filled circles) and MLF (dotted line and open circles) volleys as a function of conduction distance in the different cervical segments. The latency was measured from the onset of the stimulating pulse to the onset of the negativity (filled arrows) of the descending volley. The time of arrival in mid-C₇ of the corticospinal volley was about 1.7 (black line) and 0.5 (grey line) ms for the MLF volley.

**Motoneuronal recordings**

Intracellular recordings were made from 30 forelimb motoneurons in the lateral part of the motor nuclei (10 DR and 20 unidentified) in the C₆–C₇ segments. Figure 2A shows the antidromic action potential in a DR motoneuron (Fig. 2B; electrode track in C₇) and the effect of contralateral (Fig. 2C) and ipsilateral pyramidal (Fig. 2D) stimulation at 80 μA. In contrast to the rat (Alstermark et al. 2004), the positive potential (reversed pyramidal field potential) evoked by a single pyramidal stimulus was too small (maximum, ~50 μV) to cause the appearance of false monosynaptic excitatory postsynaptic potentials (EPSPs). Distinct EPSPs (arrows) appeared after the third contra- and ipsilateral pyramidal stimulus in this cell. Sometimes pyramidal EPSPs could be evoked from the second stimulus, as shown for another DR motoneuron in Fig. 3C. Threshold for eliciting the pyramidal EPSPs was 60–80 μA. Interaction of the ipsi- and contralateral pyramidal volleys revealed an occlusion of about 18% at 80 μA at a transverse distance of 300 μm. Thus, due to the small size of the pyramid (~250 μm), it is virtually impossible to completely exclude co-activation of fibers outside the pyramids even at threshold for evoking the EPSPs. However, since pyramidal EPSPs could only be evoked in ~15% of the tested forelimb motoneurons (in 4/28 for contralateral Pyr and 2/11 for ipsilateral Pyr), it is tentatively proposed that the late EPSPs shown in Figs. 2, C and D, and 3, A and C, are mainly evoked by stimulation of fibers in the pyramids. The low frequency of occurrence is in contrast to the rat, in which pyramidal stimulation almost invariably evoked excitation (Alstermark et al. 2004).

The latencies of the pyramidal EPSPs ranged between 3.5 and 5.8 ms, as shown in the histogram (Fig. 3E). The latencies are measured from the effective pyramidal stimulation (2nd or 3rd).

In the rat, it was shown that the pyramidal EPSPs with shortest latencies are mediated via a fast cortico-reticulospinal pathway (Alstermark et al. 2004). We therefore made a corticospinal transection in the rostral C₂ segment (Fig. 3D) and tested the effect of pyramidal stimulation, as shown for a DR motoneuron in Fig. 3, A and C. The lesion was complete (Fig. 3D) since it abolished the descending corticospinal volley on either side as shown in the cord dorsum records (Fig. 3, A and C, bottom). Nevertheless, small pyramidal EPSPs could still be evoked from both the ipsi- and contralateral pyramids with latencies of 3.8 and 3.5 ms, respectively. This finding suggests that forelimb motoneurons in the mouse, as in the rat, receive excitation via a cortico-reticulospinal pathway.

Because of the low occurrence of pyramidal EPSPs, we stimulated dorsal to the pyramids in the reticular formation (RF) and MLF. EPSPs were invariably evoked from the RF (20/20) and the MLF (21/21). Figure 3A–C shows the effect of stimulation ipsi- and contralateral in the RF and MLF for the same DR motoneuron in which weak pyramidal EPSPs could be evoked after C₂ corticospinal transection. On the contralateral side (Fig. 3C), the EPSP amplitude increased almost twice, 0.5 mm dorsal to the pyramid, which corresponds to the RF and the latency shortened from 3.5 to 2.3 ms. In the contralateral MLF, about 1.5 mm dorsal to the pyramid, the EPSP amplitude
remained rather unchanged, but the latency decreased to 1.3 ms. Further dorsally in the MLF (2.0 mm dorsal to the pyramid), the EPSPs decreased in amplitude, while the latency remained unchanged. Similar findings were obtained by ipsilateral stimulation (Fig. 3A) in the RF and MLF, except that the EPSP amplitude was smaller and that a mixture of EPSPs with latencies ~1.3 and 2.3 ms were evoked from ventral region of the MLF (+1.5 mm to the pyramid). The latencies of EPSPs evoked from the ipsi- and contralateral MLF and RF (no side difference) are shown in the histogram of Fig. 3E. Assuming that the MLF and RF EPSPs are evoked by the fast reticulospinal fibers represented by the MLF volley, the MLF EPSPs are monosynaptic and the RF EPSPs are disynaptic. These results suggest that a reticulospinal pathway may provide for the fast and strong excitatory input to forelimb motoneurons in the mouse.

**DISCUSSION**

Our results show that it is possible to achieve enough stability for in vivo intracellular recordings from forelimb motoneurons in the adult mouse to allow for investigation of synaptic effects evoked from descending systems. It will now be possible to further investigate the neuronal organization of descending systems in wild-type and transgenic mice.

The mouse, as the rat, seems to lack monosynaptic corticospinal motoneuronal excitation, but in contrast to the rat, the mouse seems to have only weak corticospinal excitation to forelimb motoneurons. This can be explained by the more medial termination in the gray matter than in the rat, avoiding the lateral part of lamina VII where many last-order interneurons are located (Alstermark and Kümmel 1990) and apparently weaker strength of the synaptic input. In the rat, large corticospinal EPSPs could be evoked, which were mediated polysynaptically via segmental interneurons (Alstermark et al. 2004).

The weak and infrequent pyramidal excitation in the mouse suggests that the corticospinal input is not primarily involved in the direct control of motoneurons, but maybe more so in the control of sensory information.

As in the rat, we found that the pyramidal excitation could be mediated via a fast disynaptic cortico-reticulospinal pathway. In the mouse, the input may come from the pyramid, but in view of the weak effect, it seems likely that other inputs like tectum may be stronger. In the rat, we know that tectum provides a strong excitatory input to the cortico-reticulospinal pathway to forelimb motoneurons (B. Alstermark, J. Ogawa, and T. Isa, unpublished results). Our results support the view, proposed by Shapovalov (1975), that “the reticulo-motoneuronal input is the most ancient direct line and its persistence in most advanced vertebrates strongly suggest its continuing importance.”

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