In vivo Recordings of Bulbospinal Excitation in the Adult Mouse Forelimb Motoneurones.

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Abstract

Here we report on pyramidal and reticulospinal excitation in forelimb motoneurones in the adult mouse using intracellular recordings \textit{in vivo}. The results have been obtained in BALB/C mice, which were anaesthetized with midazolam fentanyl/fluanison. In contrast to the rat, only weak and infrequent pyramidal excitation could be evoked with a minimal trisynaptic linkage. Disynaptic reticulospinal excitation could always be evoked as well as monosynaptic excitation from the medial longitudinal fasciculus. The results suggest that the 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 to rat. Our study provides an opening for studying the effect of genetic manipulation on specified descending systems in the mouse \textit{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 true also 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 motoneurones *in vivo* in the adult mouse. Since the work by Kuno and his colleagues (Huizir et al. 1975; Kuno 1976) on the electrophysiological properties of spinal motoneurones in normal and dystrophic mice, no publications using intracellular recordings *in vivo* can be found in Public Medline.

We have recently analysed pyramidal excitation in the adult rat using intracellular recording from forelimb motoneurones (Alstermark et al. 2003) and the work by Kuno and colleagues inspired us to attempt a similar analysis in the adult mouse. It will be shown that pyramidal stimulation evokes surprisingly weak excitation in forelimb motoneurones via the corticospinal pathway (different to rat) and that strong excitation is mediated via a fast reticulospinal pathway (similar to rat). The results suggest a partly different role in motor control of the corticospinal tract in mouse and rat.
Methods

Preparation. The results have been obtained from 15 mice (5 females and 10 males, BALB/C, from Mollegaard, Denmark,) with body weights of 24-30 g and age of 2-4 months. The animals were anaesthetized with a mixture (initial dose 0.15 ml/30 g i.p. 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 0.5mg) and Decadrone (total dose 0.4 mg) was always given (s.c.) just after anaesthesia. Ephedrine was given in doses of 0.1mg when PCO$_2$ decreased below 1% for more than 5 min. (i.p. initial dose of 0.2 ml was given 30 min. after starting anaesthesia and then repeated every 30 min.). Tracheotomy, pneumothorax and artificial respiration (rate 60/min, volume 50 ml flow) were always performed, the animals being immobilized with gallamine triethiodide (Flaxedile; 2 mg/hr i.p.). The respiratory pump was built by Staffan Berg (University of Göteborg) and consisted of a rotatory disk with a slit. Pneumothorax was carefully made using fine scissors. A 5 mm long hole was made parallel to the bones. This hole provided for 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 (kept below 500/min) and expiratory CO$_2$ were monitored continuously and kept within a physiological range. Large variations in CO$_2$ was observed (initial value around 6.0% before artificial respiration was started, which decreased to 3.0% after artificial respiration). The PCO$_2$-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 slightly stretched downwards 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 3 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 fibres were stimulated in the ipsi- and contralateral pyramids at 0.3-0.5 mm lateral to the midline, 1.5mm rostral to the obex level with a rostral angle of 30° using tungsten electrodes (100 kΩ impedance, uninsulated tips of 10 µm diameter). The threshold using 0.1 ms duration pulses was always below 10 µA and usually between 5 and 10 µA. A train of 2-4 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 boro-silicate glass micro-electrode, tip diameter around 0.5-1 µm and impedance of 10-30 MΩ, filled with 2 M-potassium citrate (pH 7.4) with a minimal membrane potential of -40 mV. Stable intracellular recording could be maintained usually between 5 and 15 minutes. The condition of the animal was good for intracellular recording between 3 and 5 hours. 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 about 300 µm. The stimulating electrodes were positioned most ventrally in the pyramids. We regularly used current strengths between 80-100 µA in order to minimise activation of fibres located outside the pyramids.

To calculate the conduction velocity of the descending volley, measurements of the distance was made in situ. The location of stimulating and recording electrodes was verified and the extent of the lesion 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 fibres in C7 was assessed by recording the extracellular field potentials evoked by a single pyramidal stimulation (contralateral side) as shown in Fig. 1 A-B. The recording tracks were spread from 15° laterally to 20° medially and covered a major part of the grey matter (A). 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 depth 200 µm from the surface and became maximal in amplitude at depths 400-600 µm in track 20° medially. In tracks 10° medially, 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 fibres 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 labelling of corticospinal fibres in the adult mouse (Isa personal communication). With respect to laminae VI-VII, the termination in the mouse differs compared to the rat, where it has been found also laterally and is larger in amplitude (Alstermark et al. 2003).

Figure 1 C shows recording from the cord dorsum of the descending corticospinal (upper row) and for comparison the medial longitudinal fasciculus (MLF; lower row) volleys (onset indicated 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 C2 to C4 was virtually identical and then decreased gradually in C5 to C7, which suggests that there was a major termination in the caudal cervical segments similar to the rat (Alstermark et al. 2003). In contrast to the rat (Alstermark et al. 2003), the volley remained rather synchronised in the caudal segments C5-C7, which indicates that the corticospinal fibres are fairly homogenous with respect to size. The conduction velocity was 8.7 m/s (SD 2.4 ms, 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. 2003). As shown in Fig. 1C (lower row) stimulation in the MLF evoked a descending volley (illustrated with faster time base), which remained virtually unchanged in amplitude from C2 to C7 and had a conduction velocity of about 50 m/s (as recently found by Tanaka et al. 2004).

In Fig. 1D, is illustrated the latency of the corticospinal (Pyr; solid line and filled circles) and MLF (dotted line and open circles) volleys as 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 ms and 0.5 ms for the MLF volley.

Motoneuronal recordings

Intracellular recording has been made from 30 forelimb motoneurones 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 (B; electrode track in C7) and the effect of contralateral (C) and ipsilateral (D) pyramidal stimulation at 80µA, respectively. In contrast to the rat (Alstermark, Ogawa, Isa; 2003), the positive potential (reversed pyramidal field potential) evoked by a single pyramidal stimulus was too small (max about 50 µV) to cause the appearance of false monosynaptic 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
motoneurone 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 (about 250 µm) it is virtually impossible to completely exclude co-activation of fibres outside the pyramids even at threshold for evoking the EPSPs. However, since pyramidal EPSPs could only be evoked in about 15 % of the tested forelimb motoneurones (in 4/28 for contralat. Pyr and 2/11 for ipsilat. Pyr), it is tentatively proposed that the late EPSPs shown in Fig. 2C-D and Fig. 3A and C are mainly evoked by stimulation of fibres 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. 2003).

The latencies of the pyramidal EPSPs ranged between 3.5 and 5.8 ms as shown in the histogram, Fig. 3E. They are measured from the effective pyramidal stimulation (2nd or 3d).
In the rat it was shown that the pyramidal EPSPs with shortest latencies are mediated via a fast cortico-reticulospinal pathway (Alstermark et al. 2003). We therefore made a corticospinal transection in the rostral C2 segment (Fig. 3D) and tested the effect of pyramidal stimulation as illustrated for a DR motoneurone in Fig. 3A 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 (bottom records in A and C). 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 motoneurones 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). Fig. 3 A-C shows the effect of stimulation ipsi- and contralateral in the RF and MLF for the same DR motoneurone in which weak pyramidal EPSPs could be evoked after C2 corticospinal transection. On the contralateral side (C), 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 (A) in the RF and MLF, except that the EPSP amplitude was smaller and that a mixture of EPSPs with latencies around 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. If assuming that the MLF and RF EPSPs are evoked by the fast reticulospinal fibres 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 motoneurones in the mouse.
Discussion

Our results show that it is possible to achieve enough stability for in vivo intracellular recordings from forelimb motoneurones 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 organisation of descending systems in the wild type and transgenic mice.

The mouse, as the rat, appears to lack monosynaptic cortico-motoneuronal excitation, but in contrast to the rat, the mouse seems to have only weak corticospinal excitation to forelimb motoneurones. This can be explained by the more medial termination in the grey matter than in the rat, avoiding the lateral part of lamina VII where many last order interneurones 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 interneurones (Alstermark et al. 2003). The weak and infrequent pyramidal excitation in the mouse, suggests that the corticospinal input is not primarily involved in the direct control of motoneurones, 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. Part of 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 motoneurones (Alstermark, Ogawa, 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".

Acknowledgements

This work was supported by a grant from the Human Science Frontier Program to BA. JO was supported by a grant from Kyorin University, Tokyo, Japan. The authors wish to thank Prof. T Isa for discussing unpublished results and G Hällström and B Dagberg for skilled histological help and Staffan Berg and Lennart Näslund for skilled craftsmanship.

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References


Legends

Figure 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. The interrupted line delineates the region of negative pyramidal field potentials. B, extracellular field potentials recorded at intervals of 200 µm from depth 200 to 800 µm from the surface. The horizontal baseline has been added. Negative field pyramidal field potentials are encircled by the interrupted black line. C, recordings made with a silver ball electrode on the dorsal column of the corticospinal volley and cord dorsum potential in mid C2 to C7 segments. The corticospinal fibres were stimulated electrically in the contralateral pyramid at a strength of 100 µA. Black arrows show the onset of the negative component of the corticospinal and MLF volleys (note the different time scales for the corticospinal and MLF volleys). D, latency of the descending volley as function of the conduction distance from the pyramidal (filled circles and solid line) and MLF (open circles and dotted line) stimulation to the segmental recording site. The arrival time in mid C7 border is indicated by the gray line for the MLF volley and by the black line for the pyramidal volley.

Figure 2. Pyramidal excitation. A, intracellular recording (upper traces) from a DR motoneurone. B, transverse section of the spinal cord in mid C7 with the remaining glass microelectrode penetrating the motor nuclei. C, the effect of contralateral pyramidal stimulation at 80 µA with a train of 3 stimuli (upper traces are intracellular and lower are from the cord dorsum). D, the effect of ipsilateral pyramidal stimulation at 80 µA with a train of 3 stimuli. E, interaction of the descending corticospinal volleys recorded in C2 and evoked from the contralateral (upper records) and
ipsilateral (middle records) pyramids and from both (lower records) simultaneously. Dotted line indicates the peak amplitude of the algebraically summated volleys and the solid line the actual summation, which was 82%. Note the faster time scale and higher amplification for the descending corticospinal volley in E compared to C and D.

Figure 3. Reticulospinal excitation. Intracellular recording from a DR motoneurone after dorsal column (DC) transection of the corticospinal tract in C2. A, the effect of ipsilateral MLF, RF stimulation with a train of 2 stimuli and pyramidal stimulation with a train of 3 stimuli at 80 µA. B, transverse section of the brain stem at 1.5 mm rostral to the Obex. The two vertical black lines indicate the stimulating electrodes and dots the stimulus positions. C, the effect of contralateral MLF, RF and pyramidal stimulation at 80 µA with a train of 2 stimuli. D, transverse section of the spinal cord in rostral C2 showing the extent of the DC transection (black line). E, latency histogram of MLF, RF and pyramidal EPSPs. Bin width is 0.2 ms. The arrival time in C7 of the descending volley in the fastest corticospinal fibres is indicated by the black arrow and for the reticulospinal fibres (stimulated in the MLF) by the grey arrow.