Lack of Monosynaptic Corticomotoneuronal EPSPs in Rats: Disynaptic EPSPs Mediated Via Reticulospinal Neurons and Polysynaptic EPSPs Via Segmental Interneurons

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Alstermark, B., J. Ogawa, and T. Isa. Lack of monosynaptic corticotr substantial somatic excitation in rats: disynaptic EPSPs mediated via reticulospinal neurons and polysynaptic EPSPs via segmental interneurons. J Neurophysiol 91: 1832–1839, 2004. First published November 5, 2003; 10.1152/jn.00820.2003. In the rat, some findings have been taken to suggest the existence of monosynaptic corticotr corticomotoneuronal (CM) connections. Because this connection is believed to be largely responsible for the ability to make independent digit movements in primates and man, it has been inferred that the monosynaptic CM connection in the rat is likewise important for skilled prehension. Comparison of intracellular recordings from forearm motoneurons in anesthetized rats, revealed no monosynaptic CM excitatory postsynaptic potentials (EPSPs). The fastest descending excitation in forearm motoneurons was disynaptically mediated via a corticoreticulospinal pathway and slowly conducted excitation via corticospinal fibers and segmental interneurons. The findings stress the importance of disynaptic corticofugal pathway to forearm motoneurons in the control of skillful digit movements.

INTRODUCTION

The monosynaptic corticotr corticomotoneuronal (CM) excitation has long been considered to be decisive in the control of independent digit movements of the hand in primates and man, and it has been proposed that such connections are the basis for the skilled forepaw control also in the rat (Liang et al. 1991). Compared with monkeys and cats, much less is known about CM connections in rodents. In the cat, motoneurons (MNs) lack monosynaptic CM excitation, and it was first shown that disynaptic pyramidal excitation is mediated via C3–C4 propriospinal neurons (PNs) and interneurons (INs) in C6–Th1 (Alstermark and Sasaki 1985; Illert and Wiedemann 1984; Illert et al. 1977; Kitazawa et al. 1993). In behavioral experiments in the cat, it has been shown that C3–C4 PNs can mediate the descending command for visually guided reaching with the forearm and that INs in C6–Th1 can mediate the command for grasping food with the forepaw (Alstermark et al. 1981). Cats have been supposed to have poor digit dexterity, but it has been shown that the cat also exhibit some degree of independent control of digits (Boczek-Funcke et al. 1998). In the macaque monkey, we also found that disynaptic pyramidal excitation can be mediated via C2–C4 PNs and emphasized its importance in the control of skilled forelimb movements both in cats and primates (Alstermark and Isa 2002; Alstermark et al. 1999). It is essential to investigate the excitatory pathways from the motor cortex to forearm motoneurons in rats to address the question about the role of the direct CM connection and skilled hand movements.

Anatomical investigations in the rat have shown that the corticospinal tract is largely crossed and that the fibers descend in the ventral part of the dorsal column (Casale et al. 1988). Minor projections of crossed and uncrossed corticospinal fibers have also been observed with the axons running in the dorsal part of the lateral funicule and medial part of the ventral funicule (Liang et al. 1991). The corticospinal fibers terminate in all laminae but most heavily in laminae IV–V (Casale et al. 1988; Liang et al. 1991). Evidence for monosynaptic connections to motoneurons was presented in a study using double labeling of the corticospinal fibers and cervical MNs, some boutons (en passant or terminaux) were found in close apposition to dendrites and somata of MNs using light-microscopic criteria (Liang et al. 1991). However, recent experiments using electron microscopy revealed no evidence for direct CM synapses in the rat spinal cord (Yang and Lemon 2003).

In the rat, excitatory postsynaptic potentials (EPSPs) can be evoked in forearm and hindlimb MNs by electrical stimulation of the contralateral pyramid, but because of the low conduction velocity of the corticospinal fibers (8–18 m/s) and temporal dispersion of the descending volley, it was not possible to make any definite conclusion regarding the synaptic linkage, although it was suggested that monosynaptic EPSPs could be mediated via slow corticospinal fibers (Bannister and Porter 1967; cf. Shapovalov 1975). Stimulation applied in the motor cortex evoked EPSPs, and the shortest linkage was considered to be disynaptic via segmental interneurons, although it was proposed that some EPSPs in a few MNs could have been monosynaptically mediated via slowly conducting corticospinal fibers (Babalan et al. 1993).

In view of the somewhat ambiguous anatomical and electrophysiological data that exist, we have now re-examined how excitation evoked by stimulation in the contralateral pyramid is transmitted to forearm MNs in the rat. It will be shown that no monosynaptic CM EPSPs can be evoked and that the minimal linkage is disynaptic. However, the most short-latency disynaptic EPSPs are not mediated by corticospinal fibers but via a...
fast corticoreticulospinal (CRS) pathway. Long-latency di- and trisynaptic excitation (mainly trisynaptic) of motoneurons is mediated by segmental interneurons in C₆–C₈ segments.

**METHODS**

**Preparation**

The results have been obtained from 42 rats (42 Wistar rats; 35 females and 7 males) with averaged body weights of 258 g (182–495) and age of 5 mo (4–12). The animals were first anesthetized with a mixture of fentanyl and midazolam (2.8 ml/kg ip) and then by α-chloralose (60 mg/kg iv). Atropin (0.5 mg), dexamethasone (2 mg), and penicillin (0.5 ml) was always given (intramuscular) just after anesthesia. Occasionally strychnine and pentobarbital sodium (Nembutal) was added in doses of 1 mg/kg and ephedrine in doses of 1 mg (intravenous). Pneumothorax and artificial respiration were always performed, the animals being immobilized with gallamine triethiodide (Flaxedil; 12 mg/h). Rectal temperature was maintained at 36–38°C, and the heart rate and expiratory CO₂ were monitored continuously and kept within a physiological range. This investigation was approved by the ethical committee of Unicam University.

A laminectomy was performed that exposed spinal segments C₁–Th₅. The deep radial (DR) and superficial radial (SR) nerves were dissected and mounted on bipolar stimulating electrodes. 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 various lesions. A posterior craniotomy was performed exposing the cerebellum and the caudal brain stem. A series of spinal cord lesions were made to selectively interrupt transmission of different descending tracts as will be explained in more detail in the result section. The dorsal column (DC) was transected at the C₅ level in six rats and at C₂ in nine rats to interrupt the transmission of the corticospinal fibers. A hemisection (without the DC) was made ipsilateral to the recording side at C₂ in nine rats to interrupt transmission of bulbospinal axons and at C₅ in two rats to interrupt transmission of bulbospinal and propriospinal axons. After the C₂ hemisection a C₅ DC lesion was made in seven rats to restrict corticospinal input to neurons in the C₁–C₄ segments (see RESULTS). A contralateral hemisection was made at C₂ in one rat. The DC and dorsal part of the lateral funiculus (DLF) were transected together at C₅ in one rat and in C₅ in one rat. All transections were made under microscope using watch makers forceps and the heart rate and expiratory CO₂ were monitored continuously. Pneumothorax and artificial respiration were always performed, the animals being immobilized with gallamine triethiodide (Flaxedil; 12 mg/h). Rectal temperature was maintained at 36–38°C, and the heart rate and expiratory CO₂ were monitored continuously and kept within a physiological range. This investigation was approved by the ethical committee of Unicam University.

**RESULTS**

**Corticospinal volley and field potential**

Figure 1A shows recording from the cord dorsal of the descending corticospinal volley evoked by electrical stimulation in the contralateral pyramid. Recording was made with a silver ball electrode positioned on the dorsal column in the C₃ DC lesion and a C₅ hemisection (without the DC) are shown in Fig. 5, E and F, respectively.

**Stimulation and recording**

Corticofugal fibers were stimulated in the contralateral pyramid at 0.5 mm lateral to the midline, 2 mm 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 <10 μA (cathodal) and usually ~5 μA. Optimal placement gave a descending volley that was maximal ~150 μA; 80% in size at 100 μA. A train of two to four stimuli given at 300 Hz and 20–200 μA was usually used. The number of stimuli of the train was always changed to ascertain which pulse was effective for evoking the synaptic effects. Recording of the descending volley was made from the surface of the DC, cord dorsal somum potential (CDP), in the middle part of the C₅–C₈ segments using a silver ball electrode. Recording was made in each segment for measurements of the conduction time of the corticospinal volley. During intracellular recording, the CDP was recorded in the same segment as the microelectrode. Intracellular records were obtained from 193 forelimb MNs in C₅–C₈ (MNs; 57 DR and 136 unidentified MNs in the same track) using borosilicate glass micro-electrodes, tip diameter ~1 μm and impedance of 3–6 MΩ, filled with 2 M-potassium citrate (pH 7.4) with a minimal membrane potential of ~40 mV. All signals were digitized, stored on hard disk (Digidata 1200, Axon Instruments, Foster City, CA) and analyzed off-line in Clampfit (Axon Instruments).

To calculate the conduction velocity of the descending volley, measurements of the distance was made in situ because we noticed that measurements made from fixed (4% buffered paraformaldehyde) tissue caused large errors due to differences in the amount of fixation. The location of stimulating and recording electrodes was verified and the extent of the lesion reconstructed from sections stained with Neutral red.

**FIG. 1.** Descending corticospinal volley and field potential. A: recordings made with a silver ball electrode on the dorsal column of the corticospinal volley and cord dorsum potential (CDP) in mid C₅–C₈ segments. The corticospinal fibers were stimulated electrically in the contralateral pyramid at a strength of 200 μA. — and •, latency measurement to the onset of the negative component N1 of the corticospinal volley; and ⋯⋯ and ⋯⋯, the latency to the peak of the negative component N2. B: extracellular recordings of field potentials evoked by pyramidal stimulation at 200 μA. The depths of recording is from the surface of the dorsal column. The angle of the electrode track was 6° lateral from the vertical line. C: latency of N1 and N2 as function of the conduction distance from the pyramidal stimulation to the segmental recording site; • and —, measurements for N1; ⋯⋯ and ⋯⋯, measurements for N2. Inset: the latencies of N2 vs. N1. The averaged arrival time at C₃/C₅ border is indicated by the red line for N1 and by the blue line for N2. D: transverse section from mid C₅ showing the electrode track used for the extracellular field recordings.
middle of the C2–C6 segments. Due to temporal dispersion, the volley became broader in shape in the caudal segments. To measure the conduction velocity of the fastest corticospinal fibers, the latency was measured from the onset of the stimulating pulse to the onset of the negativity (N1) of the descending volley as is indicated for the recording in C2. The latency for the slower corticospinal fibers was measured to the peak of the negativity (N2) of the descending volley. The peak of the negativity could only be seen down to C4 or C5 in some experiments due to the temporal dispersion at more caudal levels. In Fig. 1C is illustrated the latency for N1 (●) and N2 (○) as function of conduction distance. The inset diagram shows a high correlation of N1 versus N2 (r = 0.96). The conduction velocities ranged between 9.7 and 14.7 m/s [12.3 ± 1.8 (SD) m/s, n = 7 experiments] for N1 and 6.5 to 8.9 m/s for N2 (8.1 ± 0.9 m/s, n = 6 experiments). In Fig. 1C, the arrival on average of the corticospinal volley at the C6/C7 border is shown in red for the fastest corticospinal fibers and in blue for the slower fibers. It can be seen that on average there is a difference of ~1.3 ms.

The slow negative field potential that follows the volley is caused by the EPSPs evoked from the corticospinal fibers. By recording the extracellular field potentials as shown in Fig. 1B from mid C7, we tried to localize the termination of the corticospinal fibers in the forelimb segments. The recording track passed through the dorsal horn, lateral part of the intermediate layers and medially in the motor nuclei (Fig. 1D). A small negative monosynaptic field potential, evoked by a single pyramidal stimulation, appeared at depth 0.2 mm from the surface and became maximal in amplitude at around depth 0.8 mm as shown in Fig. 1B. At depth 1.2 mm, which corresponds to the dorsal part of the motor nuclei, the pyramidal field reversed to a positive field potential. This is in good agreement with the anatomy of the corticospinal termination (Casale et al. 1988; Liang et al. 1991). Positive monosynaptic pyramidal field potentials were recorded at depths 1.2–2.0 mm. The threshold for eliciting the monosynaptic pyramidal field potential was sometimes as low as 10–20 μA. These results show that the major termination of the corticospinal fibers is in the intermediate layers and not in the motor nuclei.

**Motoneuronal recordings**

**Intact spinal cord.** Figure 2 (A and B) shows intracellular recordings from a DR motoneuron and the effect of pyramidal stimulation at 200 and 100 μA, respectively. Similar results were observed in the unidentified forelimb motoneurons. A single pyramidal stimulus evoked a small positive potential with the time course of a monosynaptic EPSP. Distinct disynaptic EPSPs appeared after the second and third pyramidal stimuli (red arrows). Threshold for eliciting the EPSPs was near 100 μA in this cell and usually between 50 and 100 μA. Note the clear temporal facilitation with 200 μA in (A) after the third stimuli and that the EPSP consisted of two peaks: one early and one late component. Dorsoventral tracking with the stimulating electrode revealed that the effects were evoked from the pyramid. In other MNs, the early EPSP was very small or absent, whereas the late EPSP was large and had a slow rise time. These late EPSPs were usually evoked already from the second stimulus but with clear facilitation by the third stimulus. In another DR motoneuron, shown in C, a single stimulus at 100 μA evoked a large positive potential (IC; top most traces). In Fig. 1B, it was shown that the negative pyramidal extracellular field reverse to a positive potential in the motor nuclei (i.e., at depths >1.2 mm). We compared the intracellular potential with the extracellular potential recorded just outside the motoneuron as shown in Fig. 2 (A and C; EC). The averaged intracellular and extracellular recordings (C; AV) are virtually identical. Other examples are shown in Figs. 4A and 5A, respectively. We have never observed monosynaptic EPSPs in 104 tested C6–C8 MNs (33 DR motoneurons and 71 unidentified forelimb motoneurons) that could not be explained by the extracellular field and did not show temporal facilitation. Thus, it is necessary to compare the intra- and extracellular recordings if the extracellular fields evoked by the pyramidal stimulation are large.

The latencies of the pyramidal EPSPs are shown in the histogram, Fig. 2D. They are measured from the effective pyramidal stimulation (usually 2nd or 3rd). The arrival of the corticospinal volley in mid C6–C7 segments was 2.3 ± 0.15 (SD) ms for the fastest fibers (n = 7 experiments; red arrow) and 3.6 ± 0.45 ms (n = 6 experiments; blue arrow) for the slower fibers. The earliest pyramidal EPSPs had latencies of 2000 ± 300 ms if the stimulation pulse was delayed by 200 ms from the effective pyramidal stimulation. The latency of the EPSP was increased by 10.2 ± 0.3 ms if the stimulation pulse was delayed by 100 ms.

**Fig. 2.** Motoneuronal recording: intact spinal cord. A: intracellular recordings (IC; top) from a deep radial (DR) motoneuron and the effect of pyramidal stimulation at 200 μA at single, double, and triple stimuli. Extracellular recordings (EC) are shown below the CDP record. B: same cell as in A but at 100 μA. C: intracellular (top) and extracellular (2nd from top) recording from another DR motoneuron at 100 μA. Averages of the intracellular (red line) and extracellular recordings (black line) are shown (3rd from top) and the CDP (bottom). CDP recordings are shown in A and B below the intracellular records. D: latency histogram of pyramidal excitatory postsynaptic potentials (EPSPs). Bin width is 0.2 ms in this and the following latency histograms. Measurements are from the effective pyramidal stimulation. The averaged arrival time in C6/C7 of the descending volley in the fastest corticospinal fibers is indicated by the red arrow and for the slower corticospinal fibers by the blue arrow.
2.0 ms (1.8 ms in Fig. 4F), which is earlier than the arrival of the corticospinal volley in the forelimb segments. These results suggest another route for the fastest pyramidal excitation in forelimb MNs (see following text).

**SPINAL CORD LESIONS.** From studies in the cat we know that disynaptic pyramidal excitation in forelimb MNs can be mediated by C3-C4 PNs, segmental INs and reticulospinal neurons. To differentiate between these possibilities, we first made transection of the corticospinal tract in the dorsal column either in C3 or C2 or both. These lesions spared the reticulospinal pathway. In other experiments, we lesioned the reticulospinal pathways by a complete transection of the lateral and ventral funiculi, which spared the corticospinal tract in the dorsal column. Histology of a C3 DC lesion and a C2 hemisection is shown in Fig. 5, E and F, respectively. Recording was made on the side ipsilateral to the lesion/s except for the hemisection made on the contralateral side in C2 as shown in Figs. 6, B and D.

**PYRAMIDAL EXCITATION VIA RETICULOSPINAL NEURONS.** Figure 3 summarizes the results after dorsal column transection in C5 (A and B) and/or after additional transection in C2 (C and D). Qualitatively similar results were obtained if the C2 corticospinal transection was made alone or after a preceding transection in C5. The experimental arrangement is shown schematically in Fig. 3E. After the C5 and/or C2 corticospinal transections, the latencies of EPSPs ranged between 1.8 and 3.0 ms as illustrated in the histogram in F. Note the fast rise of the EPSPs and the lack of EPSPs with latencies >3.0 ms. The lesions in C3 and C2 completely abolished the direct corticospinal volley and negative cord dorsum potentials. However, as shown in Fig. 3D a synaptic volley (black arrow) was observed after the second and third pyramidal stimuli (averaged and amplified trace shown below). This volley arrived in C6-C7 ~1.5 ms after the third pyramidal stimulus. Measurement made from this volley showed EPSPs with minimal latencies in a monosynaptic range (0.3–0.9 ms) from 1.8 to 2.4 ms. The later EPSPs from 2.4 to 3.0 ms could thus have been disynaptically mediated relative to the synaptic volley. Because the first intercalated neuron must be located rostral to the C1–C2 level, it is suggested that the fastest pyramidal EPSPs are disynaptically mediated via reticulospinal neurons with axons projecting monosynaptically to forelimb MNs (for exclusion of other brain stem systems, cf. Discussion).

**PYRAMIDAL EXCITATION VIA SPINAL CORD INS.** The result of transecting the CRS pathway in C2 is shown in Fig. 4 (A–C). A schematic diagram of the experiment is shown in E. A single pyramidal stimulation evoked no EPSP as shown in Fig. 4A by comparing the intra- (top) and extracellular (bottom) records (see preceding text). EPSPs with long latencies, ≈3.2–5.2 ms (Fig. 4F), and slow rising phase were observed after the second and third stimuli as shown in Figs. 4, B and C, respectively. The large positive potential in the cord dorsum after the third stimulus was generated by an extracellular field in the motor nucleus as can be seen in the extracellular record (bottom). The field was larger than usual because strychnine had been given prior to recording. Taking the arrival time of the corticospinal volley into account (cf. preceding text), these results suggest that EPSPs with latencies starting from 3.2 to 5.0 ms could have been disynaptically mediated via the fastest and slower corticospinal fibers and EPSPs with latencies >5.0 ms could be tri- and polysynaptically mediated. It should be emphasized that EPSPs with latencies >5.0 ms was the most common finding after hemisection sparing the dorsal column (-DC) in C2 and/or C5.

**PYRAMIDAL EXCITATION VIA C3–C4 PNS.** To differentiate between effects possibly mediated via C3–C4 PNs and/or INs in the forelimb segments (below C5; denoted as segmental INs), we aimed at making lesions which spared the corticospinal input to either the C3–C4 segments or the forelimb segments C6 and caudally. Transection of the cortico-bulbospinal paths in C2 was first made to ensure that pyramidal excitation could be evoked as shown above. We then added a corticospinal transection in C4 as illustrated schematically in Fig. 4E. The only spared descending system to the forelimb segments on the ipsilateral side would be the C3–C4 PNs. In two experiments, we also added strychnine to reduce glycinergic inhibition and

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**FIG. 3.** Pyramidal excitation via reticulospinal neurons. A and B: intracellular recordings (top) from a C7 forelimb motoneuron after dorsal column (DC) transection of the corticospinal tract in C5 (CDP, bottom) and the effect of pyramidal stimulation at 100 and 200 μA, respectively. C and D: intracellular recording from a DR motoneuron after combined C5 and C2 corticospinal transections in the DC and triple pyramidal stimuli given at 100 and 200 μA, respectively. The thin black trace is the averaged record of the cord dorsum recording and has been amplified to see the synaptic volley evoked after the 3rd pyramidal stimulation. E: schematic diagram indicating the lesions and active pathway in red. In this and the following figures, solid lines indicate existing connections and dotted lines connections, which have not yet been proven or disproved. F: latency histogram of EPSPs evoked from the effective pyramidal stimulation after corticospinal transection of the DC in C5 and C2 or combined C3 and C2.
increase the excitability of synaptic transmission, but without any effect on EPSPs or fields. Intracellular recording from a DR motoneuron, after injection of strychnine, showed that no synaptic response could be evoked from the pyramid after the combined lesions in C2 and C5 (Fig. 4D). Similar results were found in 14 MNs. We also recorded extracellularly, and it can be seen in Fig. 4D that also the negative field potential evoked by pyramidal stimulation was abolished by the combined C2 and C5 lesions. This finding was confirmed in seven experiments. These results suggest that C2–C4 PNs in the rat do not mediate disynaptic pyramidal excitation in forelimb MNs in contrast to previous findings in the cat and monkey (Alstermark and Isa 2002; Alstermark et al. 1999; Illert et al. 1977). However, this finding does not exclude trisynaptic pyramidal excitation to forelimb motoneurons via C3–C4 PNs (see Discussion and Fig. 7).

PYRAMIDAL EXCITATION VIA SEGMENTAL INs. After transecting the CRS paths and propriospinal axons in C5, the only remaining descending ipsilateral input to the forelimb segments would be from corticospinal fibers to INs in the more caudal forelimb segments as outlined schematically in Fig. 5D. After this transection, pyramidal EPSPs could still be evoked and they had similar shapes and latencies (Fig. 5, A–C) as after a corresponding transection in C2 (Fig. 4, A–C). These findings suggest that disynaptic and trisynaptic pyramidal excitation in forelimb MNs can be mediated via segmental C6–C8 INs. Latencies of EPSPs are shown in Fig. 4F and were similar as after C2 hemisection (−DC).

AXONAL LOCATION OF CRS PATHWAY. To further study the axonal location of the fast disynaptic excitatory CRS pathway, we added a transection of the dorsal part of the lateral funiculus after the C2 dorsal column transection because a minor portion of the corticospinal tract is located here. As shown in Fig. 6A, distinct disynaptic EPSPs could still be evoked. This result suggests that the reticulospinal axons, which mediate the fast disynaptic pyramidal EPSPs, are located in the ventral half of the spinal cord. Because some corticospinal and reticulospinal neurons have axons that descend without crossing the midline at the brain stem level but can cross at the spinal level, we also made a hemisection of the contralateral side (to the recording

FIG. 4. Pyramidal excitation via spinal cord interneurons (INs). A–C: intracellular recordings (top) from a DR motoneuron, CDP (middle), extracellular field (bottom) and the effect of pyramidal stimulation at 200 μA after hemisection except for DC in C2. Intravenous injection of strychnine (1 mg/kg) had been given prior to the recording. D: intracellular recordings (top) from a C7 forelimb motoneuron, CDP (middle), extracellular fields (bottom) and the effect of pyramidal stimulation at 200 μA after combined C2 hemisection (except DC) and C5 DC transection of the corticospinal tract. E: schematic diagram indicating the lesions and active pathway in grey. The dotted lines indicate lack of synaptic effects. F: latency histogram of EPSPs evoked from the effective stimulus after hemisection (except DC) in C2 or C5. The averaged arrival time in C6/C7 of the descending volley in the fastest corticospinal fibers is indicated by the dark grey arrow and for the slower corticospinal fibers by the light grey arrow.

FIG. 5. Pyramidal excitation via segmental INs. A–C: intracellular recordings (top) from a DR motoneuron, CDP (middle), extracellular field (bottom) and the effect of pyramidal stimulation at 200 μA after hemisection except for DC in C5. Latencies of EPSPs are shown in Fig. 4F. D: schematic diagram indicating the lesions and active pathway in red. E: histology showing a C5 DC lesion and also the glass microelectrode. F: histology showing a C2 hemisection (except DC).
This hemisection had no effects either on the early or late EPSP components as illustrated in Fig. 6B. The latencies of the EPSPs are shown in Fig. 6C after the different lesions, and it can be seen that they were in a similar range as the EPSPs obtained after corticospinal lesions (cf. Fig. 3F).

**DISCUSSION**

**Lack of monosynaptic CM excitation**

Previous electrophysiological investigations in the rat have given different results and suggested the existence of a monosynaptic CM projection (Babalian et al. 1993; Bannister and Porter 1967; Liang et al. 1991). Our results strongly suggest that there is no monosynaptic excitatory CM connection in the rat. Because we could evoke the descending corticospinal volley with a threshold usually 5–10 μA, we also tried to evoke monosynaptic EPSPs at very weak strengths using longer pulse durations to favor activation of slow fibers and also suppressed di- and trisynaptic transmission by giving Nembutal but without any sign of monosynaptic EPSPs. Even the most short-latency EPSPs required temporal facilitation, whereas monosynaptic EPSPs should be possible to elicit by single stimulation. J. B. Nielsen, J.-M. Aimonetti, V. Marchand-Pauvert, and M. Enriquez-Denton have recorded from forelimb MNs and evoked pyramidal excitation and have likewise failed to observe monosynaptic EPSPs (but found di- and polysynaptic pyramidal EPSPs; IUPS meeting 2001, Abstract 2245). A serious problem in small animals like the rat is that extracellular field potentials are very large. If the intracellular recordings are not compared with the extracellular ones, it is possible to mistake the reversed monosynaptic pyramidal field potential for a monosynaptic EPSP. Together with recent anatomical findings based on electron microscopy (Yang and Lemon 2003), it seems safe to conclude that forelimb MNs in the rat lack monosynaptic CM connections. This clarification is important, since it has been proposed that at least part of the skilful digit movements in the rat could have been controlled by such a connection (see *Species comparison of forelimb movements*).

**Fast disynaptic excitation via CRS neurons**

The shortest latency disynaptic pyramidal EPSPs (1.8 and 2.4 ms) appeared earlier than or almost simultaneously with the direct corticospinal volley. Accordingly, it seems likely that the earliest EPSPs were mediated by another pathway than the corticospinal tract. Because these EPSPs remained unchanged after corticospinal transections (DC and DLF) either in C5 or C2 and contralateral hemisection in C2, we propose that they are mediated via reticulospinal neurons with their axons running in the ventral half of the spinal cord as illustrated schematically in Fig. 7. We can exclude the rubrospinal tract because it was lesioned with DLF transection and also the vestibulo- and tectospinal tracts because they do not receive a direct input from corticobulbar fibers (Brodal 1981). It is interesting that the CRS effects to forelimb MNs in the rat seems to be much more frequent and stronger than in the cat and macaque monkey forelimb motoneurons (Alstermark et al. 1999; Illert et al. 1977). The function of this pathway is unknown, but behavioral investigations in the rat after combined pyramidal tract and red nucleus lesions have shown that bulbospinal systems can compensate and to some extent carry the command for reaching and grasping (Whishaw et al. 1998).

**Corticospinal excitation via segmental INs**

Comparison of the histograms in Figs. 2D (intact), 3F (corticospinal lesion), and 4F (reticulospinal lesion) shows that the
We are presently recording from identified C₃-C₄ PNs in the rat and have so far not found evidence in favor of a monosynaptic corticospinal input to them (Ogawa and Alstermark, unpublished findings). Instead, we have observed that C₃-C₄ PNs can be disynaptically activated from the contralateral pyramid even after a C₂ DC transection of the corticospinal tract.

Species comparison of forelimb movements

The lack of disynaptic corticospinal excitation to forelimb MNs via C₃-C₄ PNs as well as monosynaptic CM excitation in the rat is interesting from a functional point of view. The rat possesses a rich motor repertoire especially with their forepaws, but they do not use their forelimbs as felines in hunting fast moving prey or climbing and jumping in treetops like monkeys. Rats can do reaching movement with their forelimbs, but the movement is rather inflexible if conditions suddenly change (Metz and Whishaw 2000). In this respect, the new finding with a fast disynaptic CRS pathway to forelimb motoneurons is interesting. We know that the reticulospinal neurons in the rat receive convergent excitatory input from motor cortex and tectum (unpublished results), as in the cat (Alstermark et al. 1992). Thus there is a possibility for up-dating of the descending cortical command en route to the motoneurons in a similar manner as has been demonstrated in the cat for the C₃-C₄ PNs (Illert et al. 1977). In view of the present results, we tentatively propose that the disynaptic excitatory CRS tract to forelimb motoneurons in the rat could be viewed as functionally analogous to the C₃-C₄ propriospinal system in the cat for fast visual control of forelimb movements. It is therefore interesting, that the disynaptic cortico-C₃-C₄ propriospinal pathway, first shown in the cat, recently has been demonstrated in the macaque monkey (Alstermark et al. 1999) and most likely also exists in man (Marchand-Pauvet et al. 2001). It has been proposed that such spinal premotoneuronal integration is of little or no importance in high primates and man because electrophysiological investigations in the macaque monkey failed to provide clear evidence for the existence of C₃-C₄ PNs (Maier et al. 1998; Nakajima et al. 2000). According to this view, the monosynaptic corticomotoneuronal connection has replaced the disynaptic one, and this change is correlated with the increased dexterity of digit movements in primates and man (Nakajima et al. 2000). However, the failure to frequently observe disynaptic excitation was shown to be due to a stronger inhibition of the C₃-C₄ PNs in the macaque monkey compared with the cat (Alstermark et al. 1999; B. Alstermark, Y. Ohki, and T. Isa unpublished observations in the cat). When the inhibition was reduced pharmacologically with strychnine, disynaptic cortico-motoneuronal excitation mediated via C₃-C₄ PNs could be evoked in virtually all forelimb motoneurons (Alstermark et al. 1999).

The rat as the cat has been underestimated when it comes to digit manipulation for grasping food (Alstermark and Isa 2002; Ivanco et al. 1996). Both species can do fractionated digit movements although not highly independent digit movements like the precision grip in primate. The present results suggest that the dexterity in the rat is not controlled by a monosynaptic CM connection but that skilful digit movements can be effectively controlled by di- and trisynaptic connections. In the cat, it is known that the command for grasping is disynaptically

Lack of disynaptic CM excitation via C₃-C₄ PNs

Corticospinal disynaptic EPSPs (as well as the extracellular fields) were completely abolised by adding a C₃ DC lesion (corticospinal fibers transected) after a prior C₂ hemisection (without DC; reticulospinal fibers transected). The excitability before adding the corticospinal lesion in C₃ was high as can be seen also from the CDP in Fig. 4C. The disappearance of excitation in the motoneurons was not due to strong inhibition of the intercalated neurons because even in the presence of strychnine no EPSPs or fields could be evoked. These results suggest that C₃-C₄ PNs do not mediate disynaptic corticospinal excitation to forelimb MNs in contrast to findings in the cat and macaque monkey (Alstermark et al. 1999; Illert et al. 1977). However, as outlined in Fig. 7, one possibility is that the longer-latency EPSPs (2.4–3.0 ms; Fig. 3F) after C₅ and C₂ DC transections could be trisynaptically mediated via a cortico-reticulo-propriospinal pathway to forelimb motoneurons.

FIG. 7. Excitatory corticomotoneuronal (CM) pathways. Schematic diagram summarizing the results of excitatory CM pathways, coMcx, contralateral motor cortex; Retsp, reticulospinal neuron; PN, propriospinal neuron; Segm. IN, segmental interneuron; MN, motoneuron. The neuronal circuitry represents a population of neurons, and it does not imply that every neuron makes all the indicated connections, e.g., like the corticospinal neuron. The dotted line from Retsp to C₃-C₄ PN is tentative. The white line from the coMcx to the segmental IN indicates weak synaptic connection. · · · · , connections, which have not yet been proven or disproved. The corticospinal axons are located in the DC and the reticulospinal axons in the ventral quadrant.

Latencies of EPSPs in Fig. 2D distribute over a larger time range than those in Figs. 3F and 4F. Measured from the fastest corticospinal volley, latencies >3 ms may indicate a disynaptic linkage and latencies >5 ms may suggest a trisynaptic linkage. The temporal dispersion of 1.3 ms, in the forelimb segments, of the corticospinal volley makes it difficult to indicate an exact border between di- and trisynaptic transmission. In Fig. 4F, only 6 cells had latencies in the disynaptic time range, whereas 22 had latencies suggesting a trisynaptic linkage. As outlined schematically in Fig. 7, we propose a weak disynaptic excitatory linkage together with a stronger tri- and or polysynaptic pathway. It seems likely that in so far as the intricate control of the paw in the rat depends on the corticospinal tract (Schrimsher and Reiser 1993), the trisynaptic pathway probably plays a dominating role.
mediated via the cortico- and rubrospinal tracts and segmental INs and that it can be taken over by the reticulospinal tract after complete transection of the cortico- and rubrospinal tracts (Alstermark et al. 1981; Pettersson et al. 2000). Recent observations in the macaque monkey after complete corticospinal transection in C5 show initial impairment of the precision grip, but that it returns already after 6–7 days (S. Sasaki, T. Isa, L.-G. Pettersson, B. Alstermark, unpublished observations). This finding suggests that indirect connections, from the motor cortex to forelimb motoneurons, via interneurons in the spinal cord and brain stem systems may also contribute importantly to the control of independent digit movements in all three species.

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