Motor command for precision grip in the macaque monkey can be mediated by spinal interneurons

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Alstermark B, Pettersson LG, Nishimura Y, Yoshino-Saito K, Tsuboi F, Takahashi M, Isa T. Motor command for precision grip in the macaque monkey can be mediated by spinal interneurons. J Neurophysiol 106: 122–126, 2011. First published April 20, 2011; doi:10.1152/jn.00089.2011.—In motor control, the general view is that spinal interneurons contribute mainly to reflexes and automatic movements. The question raised here is whether spinal interneurons can mediate the cortical command for independent movements, like a precision grip between the thumb and index finger in the macaque monkey, or if this function depends exclusively on a direct corticomotoneuronal pathway. This study is a followup of a previous report (Sasaki et al. J Neurophysiol 92: 3142–3147, 2004) in which we trained macaque monkeys to pick a small piece of sweet potato from a cylinder by a precision grip between the index finger and thumb. We have now isolated one spinal interneuronal system, the C3–C4 propriospinal interneurons with projection to hand and arm motoneurons. In the previous study, the lateral corticospinal tract (CST) was interrupted in C4/C5 (input intact to the C3–C4 propriospinal interneurons), and in this study, the CST was interrupted in C2 (input abolished). The precision grip could be performed within the first 15 days after a CST lesion in C4/C5 but not in C2. We conclude that C3–C4 propriospinal interneurons also can carry the command for precision grip.

digit movements; C3–C4 propriospinal system; corticomotoneuronal pathway

The ability of primates, including humans, to perform a precision grip between the thumb and index finger and independent movements of the digits is generally accepted to require the direct control of motoneurons (MNs) from the motor cortex, a connexion that has been termed the corticomotoneuronal (CM) pathway (cf. Lemon 2008). Based on electrophysiological experiments in the macaque monkey, it has been proposed (Bernhard and Bohm 1954) that excitation mediated via a monosynaptic (direct) CM pathway could be especially important in the control of fractionated digit movements. In classical behavioral studies in the macaque monkey using different lesions in the brain stem (Lawrence and Kuypers 1968a), it was concluded that the performance of highly fractionated movements of the fingers depends on the pyramidal tract and proposed that this capacity is provided by the direct CM connections (Kuypers 1982). The results suggested that also the rubrospinal tract could to some extent provide the capacity to execute independent movements (Lawrence and Kuypers 1968b) but that the direct CM pathway amplifies these effects. These studies focused on the direct CM connections, and transmission via spinal interneurons was not considered to be of importance. It was proposed that a major advantage was actually to bypass the spinal interneurons, which were considered to have too widespread connections with MNs to distal hand muscles to be able to mediate the command for independent digit movements (Kuypers 1982). However, a recent single unit recording study showed that spinal interneurons are activated and exert postspike effects in hand muscles during precision grip in monkeys (Takei and Seki 2011). In the studies by Lawrence and Kuypers (1968a, 1968b), the lesions were made in the brain stem. Therefore, it was not possible to differentiate between effects mediated via the brain stem and spinal cord. The aim of this study was to test whether or not spinal interneurons can mediate the motor command for a precision grip in the macaque monkey.

In the cat, which lacks the direct CM pathway, it has been shown that the command for visually guided reaching can be mediated via spinal interneurons denoted C3–C4 propriospinal neurons (PNs) and the command for digit grasping via segmental interneurons located in the same segments (C6-Th1) as the forelimb MNs (Alstermark et al. 1981; Alstermark and Kümmel 1990). A disynaptic excitatory CM pathway, mediated via C3–C4 PNs, was first demonstrated in the cat (Illert et al. 1977) and later also in the macaque monkey (Alstermark et al. 1999).

This study is a followup of a previous report (Sasaki et al. 2004) in which macaque monkeys were trained to pick a small piece of sweet potato from a cylinder by a precision grip between the index finger and thumb. The behavior was compared before and after a complete transection of the corticospinal tract (CST) in C4/C5 that interrupted the input to MNs and segmental interneurons in the forelimb segments (C6-Th1). In three monkeys, it was found that the precision grip and also, to some degree, independent digit movements could be observed already within the first to third weeks (Sasaki et al. 2004). However, it could not be concluded whether the command for the remaining movements was mediated via bulbospinal systems originating in the brain stem or via interneuronal systems in the spinal cord above the C4/C5 level.
In this study, we have made CST lesions in C2 and compared the preoperative performance with the initial (first 15 postoperative days) effects on grasping. Comparison of the new results with those obtained after C4/C5 CST lesions (Sasaki et al. 2004) suggests that C3-C4 PNs can mediate the command for the precision grip.

MATERIALS AND METHODS

Behavioral test. Two female monkeys (Macaca fuscata; body weight: 5.2 and 5.1 kg) were trained to retrieve a morsel of food from a horizontal tube positioned in the midsagittal plane, at the shoulder level, and at a sagittal distance of 15 cm. Each experiment (30 min) consisted of ~100 trials. In each trial, the animal retrieved, with a precision grip, a morsel of food positioned on a pin inserted through the bottom of the tube. The movements were filmed with standard video cameras (33 frames/s) positioned above, below, and on the side of the performing limb. Movies of the results shown in Figs. 3 and 4 are available in the Supplemental Material.1

Surgery. Animals were first anesthetized with ketamine (0.3 ml) and xylazine (0.6 ml) and then with Nembutal (20 mg/kg). The segmental border at C2 was exposed by a laminectomy, and a transverse durotomy was made. The CST lesion was made under a surgical microscope using watchmaker’s forceps as previously described in detail by Sasaki et al. (2004). The lesions were aimed at the smallest size giving complete interruption of the monosynaptic CM excitatory connection. The descending volley was not monitored.

Electrophysiological experiments. After the behavioral test period (survival time: 7.5 mo for monkey M and 17 mo for monkey H), the animals were first anesthetized with ketamine (0.3 ml) and xylazine (0.6 ml), and, after the tracheotomy, isoflurane (1.0–2.0%) was used throughout the surgery. After surgery, the anesthesia was changed to α-chloralose (75–100 mg/kg). Blood pressure was maintained around 100 mmHg, and Pco2 was maintained at around 4.0%. A drip of Ringer-glucose solution was given during the entire experiment, and the urinary bladder was emptied regularly. Atropin (0.5 mg), decadron (4 mg), and gentacine (1 ml) was given just after anesthesia. Atropin was given at intervals of 4–5 h. The animals were paralyzed with pancuronium bromide (1 ml, 0.2 mg/ml) given at 30-min intervals and artificially ventilated with a pump. A pneumothorax was made just before the intracellular recording.

A craniotomy was made, which exposed the posterior part of the cerebellum and the caudal brain stem to place the pyramidal electrode. It was calibrated at the obex (angle: 65° from the vertical line) and placed ~2.5 mm rostrally, 1.25 mm laterally, and at a depth of 5.0 mm from the bottom of 4Vth ventricle. The threshold for eliciting the descending pyramidal volley was usually around 5 μA. Monopolar cathodal pulses (0.1-ms duration) were applied using tungsten electrodes with an impedance around 50–100 kΩ. A laminectomy was made of the C2-Th1 and Th6–10 segments. The deep radial nerve was dissected and mounted in a cuff with creyl violet staining.

RESULTS

Electrophysiology. Because it is not possible to determine if the CST lesions in C2 are complete or not only from the histology (see Fig. 2D), we made acute electrophysiological control exper-

Supporting information. The experiments were subjected to prior ethical reviews by the Ethical Committee of the National Institutes of Natural Sciences and were performed in accordance with the guidelines of the Ministry of Education, Culture, Sports, Science and Technology of Japan and National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Online movie. A movie is available showing the results of Figs. 3 and 4 in the Supplemental Material.
We also recorded intracellularly from forearm and hand MNs in the C6-Th1 segments. The experimental arrangement is shown in Fig. 2E. For simplicity, only the direct and indirect pathways via C3-C PNs are indicated. Recordings were made from 30 MNs on the lesioned side and, for comparison, from 24 MNs on the intact side. No monosynaptic CM excitation could be evoked in the MNs on the lesioned side in any of the monkeys after the unilateral corticospinal lesion made in C2 (monkeys M and H).

The effect of a CST lesion at the level in C2 is shown in Figs. 2, which shows intracellular recordings (uppermost traces) from two different deep radial MNs obtained from monkey M after single (A) and double (B) stimulations of the contralateral pyramid. The arrow in Fig. 2A indicates the monosynaptic EPSP (gray trace) on the intact side evoked by a single stimulation in the contralateral pyramid. On the lesioned side, no monosynaptic EPSP (black trace) could be elicited, only an EPSP with long latency. Figure 2B shows that a double stimulation in the contralateral pyramid evoked a monosynaptic EPSP (arrow) and a disynaptic EPSP (interrupted vertical line) on the intact side, whereas only a disynaptic EPSP was evoked on the lesioned side. Excitatory effects in MNs were observed in 100% on the intact side versus 77% on the lesioned side. The segmental latencies of EPSPs evoked from MNs on the intact side (gray bars) and on the lesioned side (black bars) after C2 CST lesions are shown in Fig. 2C.

Monosynaptic EPSPs were in the range of 0.6–1.0 ms as measured from the incoming corticospinal volley on the intact side. The shortest latencies for disynaptic EPSPs were from 1.1–1.2 ms. Since the CST lesions in C2 were complete, these results show that di- and trisynaptic pyramidal EPSPs can be mediated via bulbospinal neurons.

Behavior. Preoperatively, the precision grip between the thumb and the index finger predominated and was used in 85–97% of the trials in the different animals. Representative preoperative movements are shown in Fig. 3A (monkey H) and in the online movie (see the Supplemental Material). Note the preshaping of the digits before the insertion into the tube (frame 2). A precision grip of the morsel was then formed by flexion of the index finger combined with an apposition movement of the thumb (frames 3–5). By continuing these movements, the morsel was removed from the pin (frame 6), and,
after withdrawal from the tube, it was held between the distal phalanges of the index finger and the thumb (frame 7).

The effect of a CST lesion at the level of C2 is shown in Fig. 3, B–D, and in the online movie (see the Supplemental Material). As soon as the animals were able to insert their digits into the tube, it was evident that digit grasping was completely abolished. Figure 3D shows the representative behavior of monkey M on postoperative day 9. The digits are evidently paretic and just pushed with the dorsum against the morsel. In monkey H (Fig. 3B; postoperative day 5), the digits were more extended during the insertion so that the palmar side of digits 2–4 could be positioned on the morsel (frame 4) but without any sign of digit flexion (frames 5 and 6). The morsel was raked out from the tube and lost (frame 7). Digit flexion improved successively so that at the end of the second postoperative week, the morsel could be taken in 60% and 50% of the trials in monkeys H and M, respectively. However, as shown in Fig. 3C (monkey H, postoperative day 15), the morsel was not taken by a precision grip. Instead, both animals invariably used a power grip, i.e., grasping of the morsel with digits 2–4 and holding it with the clenched hand. A partial recovery of the precision grip started after 3 mo and then reached a success rate of 25–30%.

Figure 4, A and B, shows stick figure representations of six movements preoperatively and on postoperative days 6 and 15 (monkey H). Figure 4A shows the last frame before the onset of the removal of the morsel from the pin, and Fig. 4B shows the first frame after the removal of the morsel from the tube. Blue lines represent the index finger and red lines represent the thumb. In contrast to the precision grip invariably observed preoperatively (first column), the movements on postoperative day 6 showed only slight flexion of the index finger. Note that only the middle phalanx of the index finger was in contact with the morsel with the result that it was pushed off the pin and dropped outside the tube. On postoperative day 15, the morsel was grasped by using flexion at the interphalangeal joints of the thumb, forming a power grip with the morsel in contact with the proximal as well as the distal phalanx of the thumb. Similar results were obtained in monkey M. Figure 4, C–F, shows the ability to move digits independently preoperatively and the loss of such movements after the lesion (monkey H). The stick figures in Fig. 4, C and D, represent the orientation of the second and third digits (5 movements superimposed) at the end of reaching when the morsel was approached. It is evident that preoperatively (C) the index finger is less flexed than the third digit. Postoperatively, note the lack of flexion in both digits (D). Figure 4, E and F, shows representative images of the hand during the movement of manual transport of the morsel to the mouth, which succeeded the digit grasping. Preoperatively (Fig. 4E), digits 3–5 were flexed, whereas digits 1 and 2 were extended and used for the precision grip. Postoperatively (Fig. 4F; postoperative day 15), the morsel was held with flexion of all digits.

**DISCUSSION**

The behavioral results revealed a striking difference in the ability to perform the precision grip after transection of the CST in C4/C5 versus C2. We (Sasaki et al. 2004) have previously shown that the precision grip completely recovers already within the first 2 postoperative weeks after a minimal but complete transection of the CST in C4/C5. In contrast, the precision grip was completely lost in monkeys M and H after CST transection in C2, whereas the power grip returned already after 2 wk. We conclude that the command for the precision grip can be mediated via C3-C4 PNs already within the first 2 postoperative weeks but not via bulbospinal neurons, which instead can mediate the command for a power grip. Thus, our findings do not support the view that the monosynaptic CM pathway is solely responsible for the control of fine digit movements but show that a spinal interneuronal pathway, the C3-C4 PN system, can effectively contribute to this control.
However, our results do not invalidate the importance of the monosynaptic CM pathway and of segmental interneurons in the C6-Th1 segments (Takei and Seki 2010). We do not exclude that the monosynaptic CM pathway may be critical for more complex finger movements than tested in this study. Taken together, our present findings confirm the work of Lawrence and Kuypers (1968a) demonstrating that the control of highly fractionated finger movements depends on the pyramidal tract but, in addition, show that this control can be mediated via a group of spinal interneurons and not via bulbospinal neurons.

The finding that bulbospinal neurons can mediate the command for the power grip is in agreement with previous work (Lawrence and Kuypers 1968a, 1968b). Monosynaptic reticulospinal excitation in forelimb MNs has been recently demonstrated (Riddle et al. 2009). It is unlikely that our C2 lesions spared the rubrospinal tract, because of similar axonal location as the CST. In the present control experiments (monkeys M and H), we did not test for rubrospinal effects, because we know from acute experiments that these lesions are in fact larger than necessary for abolishing the rubrospinal volley. We tested for effects from the ipsilateral pyramid in the acute control experiments and found oligosynaptic excitation in some MNs. The amplitudes of these ipsilateral pyramidal EPSPs were very small, and the fibers of the uncrossed ipsilateral CST were also intact after the C4/C5 CST lesions, as previously published (Sasaki et al. 2004). Thus, even if a contribution from the spared and uncrossed CST located in the ventral funiculus cannot be completely ruled out (Nishimura et al. 2009), a major contribution from this system seems less likely at present.

Our electrophysiological experiments revealed changes in transmission in nonmonosynaptic CM pathways after the CST lesions. In monkeys without chronic lesions, there is a strong inhibitory control of C3-C4 PNs (Alstermark et al. 1999), and disynaptic pyramidal excitation can only be recorded in a small percentage of MNs. If glycinergic inhibition was blocked by an intravenous injection of strychnine, disynaptic EPSPs were found in virtually all MNs (Alstermark et al. 1999). In the present control experiments (monkeys M and H), disynaptic excitation could be invariably evoked (100%, 12 of 12 cells) on the intact side without the usage of strychnine (we never gave strychnine in these experiments). It has been postulated that there is a downregulation of the inhibition after the chronic C4/C5 CST lesion (cf. Sasaki et al. 2004). This interpretation is supported by experiments in stroke patients, in which an enhancement of excitation mediated via PNs was also observed (Mazevet et al. 2003). In the present study, di- and trisynaptic EPSPs were regularly found also after chronic C2 lesion (75%, 15 of 20 cells). In contrast, after an acute lesion at the same level, disynaptic EPSPs were much less frequent (8%) despite the blockade of glycinergic inhibition with strychnine (Alstermark et al. 1999). We infer that the chronic C2 lesion induced plastic changes in corticoreticulospinal pathways on both the intact and lesioned sides.

The present findings also pertain to the phylogenetic development of spinal interneuronal pathways. In the cat, the C3-C4 PN system normally mediates the command for reaching but not for digit grasping (Alstermark et al. 1981). The contribution of C3-C4 PNs to digit movements in primates suggests that a new function has been added to this premotorneuronal system during evolution. The advantage of linking the control of digit grasping to that of reaching is apparent in primates, including humans, e.g., when grasping small objects or during object transport, when reaching may be combined with a precision grip. In the primate, including humans, the control of prehension is commonly discussed in relation to cortical mechanisms. It is interesting that a coupling between reaching and grasping may, at least partly, be controlled at the level of the spinal cord.

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