Contribution of propriospinal neurons to recovery of hand dexterity after corticospinal tract lesions in monkeys

Takamichi Tohyama[a,b,1,2], Masaharu Kinoshita[c], Kenta Kobayashi[d,e], Kaoru Isa[a], Dai Watanabe[f], Kazuto Kobayashi[g], Meigen Liu[b], and Tadashi Isa[a,e,h,3]

[a]Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan; [b]Department of Rehabilitation Medicine, Graduate School of Medicine, Keio University, Tokyo 160-8582, Japan; [c]Department of Physiology, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan; [d]Section of Viral Vector Development, National Institute for Physiological Sciences, Okazaki 444-8585, Japan; [e]The Graduate University for Advanced Studies (SOKENDAI), Hayama 240-0193, Japan; [f]Department of Molecular and System Biology, Graduate School of Biostudies, Kyoto University, Kyoto 606-8501, Japan; [g]Department of Molecular Genetics, Institute of Biomedical Sciences, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan; and [h]Department of Neuroscience, Graduate School of Medicine and Faculty of Medicine, Kyoto University, Kyoto 606-8501, Japan

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The direct cortico-motoneuronal connection is believed to be essential for the control of dexterous hand movements, such as precision grip in primates. It was reported, however, that even after lesion of the corticospinal tract (CST) at the C4–C5 segment, precision grip largely recovered within 1–3 mo, suggesting that the recovery depends on transmission through intercalated neurons rostral to the lesion, such as the propriospinal neurons (PNs) in the midcervical segments. To obtain direct evidence for the contribution of PNs to recovery after CST lesion, we applied a pathway-selective and reversible blocking method using double viral vectors to the PNs in six monkeys after CST lesions at C4–C5. In four monkeys that showed nearly full or partial recovery, transient blockade of PN transmission after recovery caused partial impairment of precision grip. In the other two monkeys, CST lesions were made under continuous blockade of PN transmission that outlasted the entire period of postoperative observation (3–4.5 mo). In these monkeys, precision grip recovery was not achieved. These results provide evidence for causal contribution of the PNs to recovery of hand dexterity after CST lesions; PN transmission is necessary for promoting the initial stage recovery; however, their contribution is only partial once the recovery is achieved.

spinal cord injury | plasticity | neural circuit | nonhuman primates | viral vector

As a potential treatment for brain and spinal cord injury, regeneration of the injured axons has been attempted in animal models, often using rodents with corticospinal tract (CST) lesions. Regeneration of the injured CST fibers was facilitated by treatments such as peripheral nerve graft (1), application of an antibody against neurite growth inhibitors IN-1 (2), combined application of the antibody and neurotrophic factor NT-3 (3), and transplantation of neural stem cells derived from induced pluripotent stem cells (4). A more recent study showed that regenerated CST axons increased the connection to the spinal motoneurons (MNs) after spinal cord injury in monkeys (5). However, in these studies, the causal contribution of such regenerated fibers to the functional recovery was not directly demonstrated. Therefore, recent debates address the question whether these therapies should target repairing the injured CST fibers and/or facilitating compensation by indirect cortico-motoneuronal (CM) connections via other descending motor pathways (6–9).

Traditionally, the neural control of dexterous hand movements in higher primates has primarily been associated with development of the direct pathway from the motor cortex to MNs, known as the CM pathway (10–12). Lesion of the CST at the brainstem level in nonhuman primates caused near-permanent loss of dexterous hand movements (13). The authors also argued for partial compensation of grasping movements by the brainstem-mediated descending pathways such as the rubrospinal tract (14). More recent studies on the neural basis of functional recovery following CST lesions revealed that a variety of plastic changes in neural circuits occurred in the supraspinal structures including the motor and premotor cortices (15), and in the connectivity from the nucleus accumbens to motor cortex (16, 17). At the more caudal level, sprouting of the midline-crossing CST fibers was revealed in the lower cervical segment of monkeys with hemisected spinal cords (18). In addition, the "indirect" CM pathways via interneurons such as the propriospinal neurons (PNs), which are located in the midcervical segments and project to hand/arm MNs in the lower cervical segments, and/or reticulospinal neurons (RSNs) in the pontomedullary reticular formation might also be involved in mediating cortical commands to MNs of forelimb muscles in animal models with CST lesions (19–21). To demonstrate which pathways causally contribute to recovery after damage to the CST, selective and reversible manipulation of particular pathways is needed.

Significance

There are different views about the targets of regenerative therapies to induce functional recovery in patients with motor paralysis following brain and spinal cord injury: whether we should aim at repairing the injured corticospinal tract or at facilitating compensation by other descending motor pathways. To help answer this question, we used double viral vectors to reversibly and selectively block the propriospinal neurons (PNs), one of the major intercalated neurons mediating cortical commands to motoneurons, in monkeys with partial spinal cord injury. We demonstrated causal roles of the PN-mediated pathway in promoting recovery of hand dexterity after the lesion. Thus, targeting the PNs might lead to developing effective treatment to facilitate recovery after spinal cord injury.

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[1]Present address: Division of Cerebral Integration, Department of System Neuroscience, National Institute for Physiological Sciences, Okazaki 444-8585, Japan.

[2]Present address: Department of Rehabilitation Medicine, Keio University School of Medicine, Tokyo 160-8582, Japan.

[3]To whom correspondence should be addressed. Email: isa.tadashi.7u@kyoto-u.ac.jp.

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Historically, much less attention has been paid to the "indirect" CM pathways in the control of dexterous hand movements compared with the direct CM pathway, because they were supposed not to be involved in the control of fine digit movements. During the last decade, however, lesion and electrophysiological studies in nonhuman primates suggested that the pathway through the PNs might be involved in the control of dexterous hand movements in the intact state (21, 22). However, this suggestion was based on the indirect evidence obtained by comparing the behavioral effects caused by transection of the CST at different rostrocaudal levels. Recently, however, a technique was developed that can selectively and reversibly control transmission via neurons comprising a particular pathway using two different kinds of viral vectors and doxycycline (Dox)-inducible tetanus neurotoxin. Applying the technique to the PNs in intact monkeys revealed that the PN-mediated pathway could carry the command for dexterous hand movements in the intact state (23). However, contribution of the PN-mediated pathway to recovery after lesion of the direct CM pathway in the monkey remains unclear. In the present study, to examine whether the PNs causally contribute to recovery of dexterous hand movements after damage to the CST, we applied the pathway-selective and reversible blocking method to the PNs in monkeys after lesioning the CST at C4–C5.

Results

The experiments were conducted on six macaque monkeys (4.0–6.0 y, 4.0–8.3 kg; see details in Table S1). Two kinds of viral vectors were injected into the cervical spinal cord of each monkey (Fig. 1*A*). The first was a highly efficient retrograde gene transfer vector, HiRet (24) or its derivatives [FuG-E (25) or NeuRet (26)], carrying the sequences for tetracycline-inducible, enhanced GFP (EGFP)-tagged enhanced tetanus neurotoxin light chain (eTeNT) (27). This vector was injected into the ventral horn of the C6–Th1 segments, where MNs innervating distal forelimb muscles are located. The second was the adeno-associated viral (AAV) vector carrying the sequence for the highly efficient Tet-on, rtTAV16. This vector was injected into the intermediate zone of the caudal C2 to caudal C4 segment, where the cell bodies of PNs are located, thus enabling the Dox-inducible blockade of PNs (23). CST lesions were then made between the injection sites of the two viral vectors, mostly in C4–C5 (Fig. 1 *A* and *B*). Two different sets of protocols for administering Dox were arranged (Fig. 1*C* and Fig. S1) to determine whether and how the PNs contributed to recovery of dexterous hand movements after the lesion, as described below. At the end of the behavioral observation, acute electrophysiological experiments were conducted under anesthesia to confirm the extent of the lesion and the effects of blockade of PNs by the vectors.

Confirmation of the Extent and Completeness of Lesion. The dorsolateral funiculus was lesioned unilaterally at the C4–C5 segment to completely interrupt the direct CM transmission (15, 17, 19, 21, 28, 29), while sparing the axons of the PNs and RSNs. In the present study, five of the six monkeys had a lesion in C4–C5 (monkeys U, B, S, K, and R), whereas the lesion of monkey N was located in C6/C7 (Fig. 1*B* and Table S1). The extent of the lesions was 42.5–70.4% of the whole lateral and ventral funiculi (*SI Materials and Methods* and Table S1). In monkeys S and N, the lesions partly extended to the dorsal column. Completeness of the lesion was examined electrophysiologically by recording the cord dorsum potentials at C6 and extracellular field potentials in the deep radial motor nuclei at C6–C7, that is, caudal to the CST lesion, evoked by stimulation of the contralateral pyramid (Fig. 2*G*). No direct volleys or monosynaptic negative field potentials were found in the affected side, which was the side with the viral vector injections and a chronic spinal cord injury, in any of the monkeys examined (a representative is shown in Fig. 2*H*), revealing that the lesions completely abolished the direct CM connections.

Visualization of "Blocked" PNs. The double-infected PNs were visualized with anti-GFP immunohistochemistry (Fig. 2 *A*–*F* and Fig. S2) (23). Labeled cell bodies were located mainly in the lateral portions of laminae VI–VII of Rexed (Fig. 2 *A*–*D* and Fig. S2), that is, the intermediate zone where PNs are known to be located (30). Their longitudinal distribution extended from C2 to the rostral part of C6 (Fig. 2*E* and Fig. S2). As described above, the CST lesion was located mainly in C4–C5. Therefore, some GFP-labeled cells were located caudal to the lesion in two monkeys (U and R), presumably due to spread of the vector solution beyond lesion sites. However, we consider that this

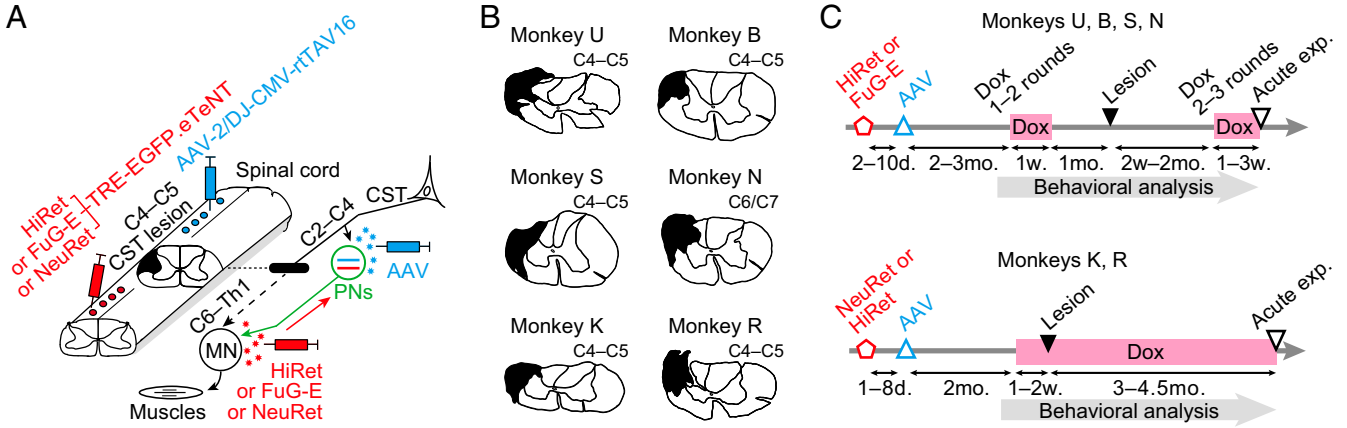

Fig. 1. Experimental procedures and the extent of lesions in all of the six monkeys. (*A*) We injected a retrograde gene transfer vector, HiRet/FuG-E/NeuRet-TRE-EGFP.eTeNT, into C6 to Th1 and successively injected an anterograde vector, AAV-2/DJ-CMV-rtTAV16, into caudal C2 to caudal C4, that enabled doxycycline (Dox)-inducible blockade of propriospinal neurons (PNs) in the midcervical segments (23). The lateral corticospinal tract (CST) was then lesioned between the injection sites of the two viral vectors, mostly in C4–C5 (*Left*, in black; *Right*, black bar). MN, motoneurons. (*B*) Lesions are indicated by black areas. (*C*) Two different sets of protocols for administering Dox (pink bars) were arranged. In the transient–PN-blocked group (monkeys U, B, S, and N), 2–3 mo after the viral injections (HiRet or FuG-E, red open pentagon, and AAV, blue open triangle), administration of Dox for 1–3 wk was repeated one to three times before and after the lesion (black filled triangle). In the continuously PN-blocked group (monkeys K and R), 2 mo after the viral injections (NeuRet or HiRet, red open pentagon, and AAV, blue open triangle), administration of Dox from 1 to 2 wk before lesion continued for 3–4.5 mo after the lesion. Black open triangles, terminal acute electrophysiological experiments.

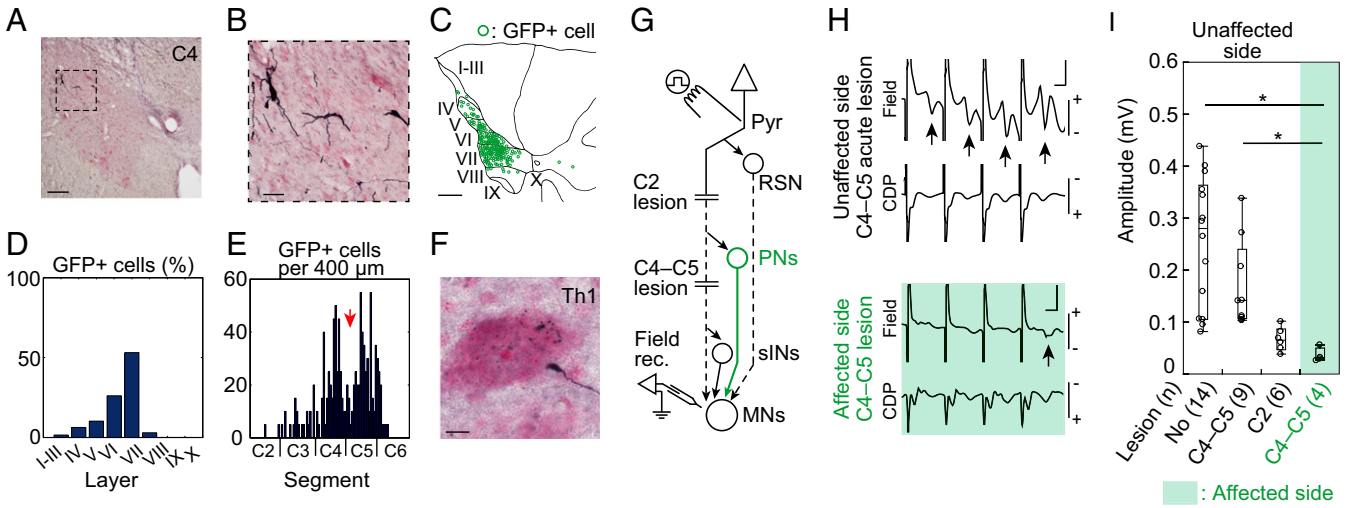

Fig. 2. Visualization of blocked PNs and confirmation of blockade of synaptic transmission through PNs. *A–F* were obtained from monkey U, and *G–I* were obtained from monkey K. (*A*) Representative GFP-labeled cells in C4. Dashed square indicates area shown in *B*. (Scale bar, 200 μm.) (*B*) High magnification of *A*. (Scale bar, 50 μm.) (*C*) Reconstructed distribution of GFP-labeled cells by multiple sections, which were separated by 200 μm. I–X, laminae of Rexed. (Scale bar, 500 μm.) (*D*) Distribution of GFP-labeled cells in individual laminae (I–X). (*E*) Longitudinal distribution of GFP-labeled cells along the spinal cord. Red arrow indicates the site of lesion. (*F*) A representative axon and bouton of GFP-labeled cell in a motor nucleus at Th1. The sections were counterstained with Neutral Red. (Scale bar, 10 μm.) (*G*) The arrangement of terminal acute electrophysiological experiments. In addition to the direct cortico-motoneuronal connection, indirect pathways through reticulospinal neurons (RSN), propriospinal neurons (PNs), and segmental interneurons (sINs) might exist. During the experiments, the lateral CST was transected at C4–C5 and successively at C2. MNs, motoneurons. Pyr, contralateral medullary pyramid. (*H*) Representative field potentials (Field) and cord dorsum potentials (CDP) in the unaffected side of a monkey with a C4–C5 acute lesion (*Top*) and in the affected side (*Bottom*) following four trained stimuli of Pyr at 200 μA. Arrows indicate disynaptic field potentials. (Vertical scale bar, 0.2 mV; horizontal bar, 1 ms.) (*I*) Quantitative analysis of the amplitudes of the disynaptic field potentials with no lesion, a C4–C5 lesion, and a C2 lesion on the unaffected side, and with a C4–C5 lesion on the affected side. The numbers of records are shown in parentheses. The box plots represent the 25th and 75th percentiles of the data. *$P < 0.01$ (the Wilcoxon test).

would not affect the main conclusion of this study (*Discussion*). Axons and boutons of GFP-labeled cells were found in the motor nuclei at C6–Th1 (Fig. 2*F*), suggesting that the blocked cells were directly connected with MNs innervating distal forelimb muscles.

Confirmation of Blockade of Synaptic Transmission Through PNs. We examined to what degree the PN-mediated cortical commands to MNs were blocked by the administration of Dox by recording extracellular field potentials in many locations of the deep radial motor nuclei, each separated by more than 200 μm, in response to stimulation of the contralateral pyramid (Fig. 2*G*) under anesthesia. To dissociate effects of the PN-mediated pathway from other indirect pathways that could mediate the cortical commands to MNs, such as those through segmental interneurons (sINs) and RSNs, the lesions of the dorsolateral funiculus were made sequentially at the C4–C5 level on the unaffected side and then at the C2 levels on both sides during the acute experiments (Fig. 2*G*). Strychnine was injected i.v. to reduce glycinergic inhibition from the CST. The amplitudes of the disynaptic field potentials in the affected side were significantly smaller than those in the unaffected side after acute C4–C5 CST lesion on the unaffected side (Fig. 2 *H* and *I*, and Fig. S3), suggesting that synaptic transmission through the propriospinal and reticulospinal pathways in the affected side were much weaker compared with those in the unaffected side. After additional C2 CST lesion on the unaffected side (Fig. 2*I* and Fig. S3) and on the affected side (Fig. S3), the amplitudes were very small, suggesting that contribution of the reticulospinal pathway was minor in both sides. These findings revealed that synaptic transmission presumably through the PNs was blocked by 74.2–93.3% [mean (SD), 83.1 (8.2)] compared with the unaffected side in four monkeys (U, S, N, and K), whereas no significant blocking was observed in monkey B, as will be discussed later. No data were obtained from monkey R due to a sudden change in the monkey's condition during the experiment.

Partially Impaired Recovery of Dexterous Hand Movements After the CST Lesion by Transient Blockade of PNs. We conducted behavioral observations of recovery time courses after the CST lesions using a reach and grasp task (Fig. 3 and Movies S1–S4). The monkeys were trained to reach for and grasp a piece of sweet potato presented on the other side of a slit (8- to 10-mm width) with a precision grip, which was defined as a grip using just the index finger and the pad of thumb (the first and third rows of Fig. 3*A*, the *Left* of Fig. 3*B*, the first row of Fig. 4*A*, the *Left* of Fig. 4*B*, and Movies S1 and S3). Before lesion, administration of Dox partly and transiently impaired these dexterous hand movements, such as precision grip, correctness of gripping, and reaching, consistent with a previous study (23). During administering Dox, success rate (*SI Materials and Methods*, *Behavioral Testing*) dropped by 15.8–34.1% in transiently PN-blocked monkeys (U, B, S, and N). One to 2 mo after lesion, administration of Dox impaired dexterous hand movements that had already shown recovery, for instance, in cooperative movements of the index finger and thumb (Fig. 3 *A* and *B*, and Movies S1–S4). Decreased success rates generally tended to start 1–2 d after the start of Dox administration and returned to baseline levels within 1 wk, even after lesion, suggesting a limited role of PNs in dexterous hand movements in the intact and once-recovered state. Recovery time courses differed between the following two groups. In the well-recovered group, including monkeys U and B, dexterous hand movements recovered within less than 1 mo after the lesion. On the other hand, in the partial-recovery group (monkeys S and N), there was no full recovery during the entire observation period even through intensive rehabilitative training, probably because of the lesion in C6/C7 (monkey N, Fig. 1*B*, and Table S1) and extended lesions to the dorsal column (monkeys S and N, Fig. 1*B*) (31) and/or to the ventral part of the lateral funiculus (monkey S; the extent of lesion was 70.4%; Fig. 1*B* and Table S1). Despite different recovery time courses in these two groups, success rates for recovered or partially recovered

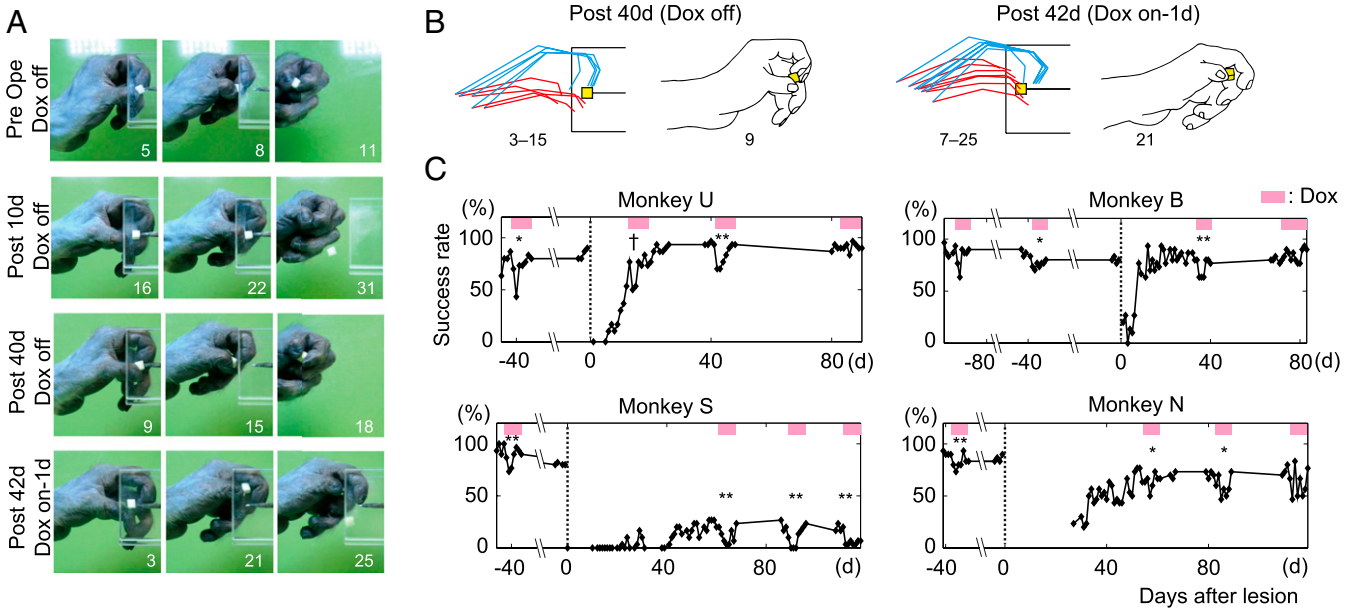

Fig. 3. Behavioral effects following transient blockade of PNs after the CST lesion. (*A*) Recovery process of dexterous hand movements 10 and 40 d after the CST lesion and a typical example obtained 42 d after the lesion (1 d after start of Dox) in monkey U. The numbers below in each panel indicate frame numbers (30 frames/s) from the moment when the digit passed through the edge of slit. (*B*) Stick diagram and drawing of grasping movements of monkey U. Blue line, the index finger and estimated second metacarpal. Red line, the thumb and estimated first metacarpal. Every third frame before and after the time of touching a piece of food to just before pulling it out in a single trial are superimposed at 40 d (Dox-off) and at 42 d (Dox-on, 1 d after start of Dox) after lesion. The numbers below indicate frame numbers from the moment when the digit passed through the edge of slit. (*C*) Recovery curves of success rate for dexterous hand movements (*SI Materials and Methods*, *Behavioral Testing*) after lesion in four monkeys (U, B, S, and N). The data are aligned to the day of lesion (dashed line). Pink bars, administration of Dox. $^{*}P < 0.05$, $^{**}P < 0.01$ (comparison of total success rate for 4 d before and after Dox using the Pearson χ^2 test). $^{\dagger}P < 0.05$ (comparison of total success rate for 2 d before and after Dox using the Pearson χ^2 test).

dexterous hand movements decreased significantly during Dox administration 1–2 mo after lesion (success rates dropped by 19.1–24.1% in the four monkeys). These results provided evidence for partial, but causal contribution of the PNs to recovery of dexterous hand movements after the CST lesion. In contrast, the effects of Dox administration 2–3 mo after lesion differed between the two groups; in the well-recovered group (monkeys U and B), no significant decrease in success rates were observed during administration of Dox 71–87 d after lesion, whereas in the partial-recovery group (monkeys S and N), success rates were still significantly decreased 82–92 d after lesion. These findings suggest that PNs contribute to recovery in different ways depending on the stage of recovery, which might vary depending on the severity of the lesions. It is worth remembering that electrophysiological analysis indicated that neurotransmission through the PNs was not obviously blocked in monkey B (Fig. S3). In this monkey, the behavioral effects of Dox were relatively weak, likely due to low infection and/or expression rate of the viral vectors, as the number of GFP-labeled cells was small (Fig. S2). It was also supposed that expression of eTeNT might have deteriorated during the long survival time after the viral vector injection (255 d; see table in Fig. S1).

Impairment of Recovery Under Continuous Blockade of PNs. In monkeys K and R, Dox was administrated continuously to suppress PNs over the entire time course of recovery after the lesion. These monkeys could not perform the precision grip (Fig. 4*C*, squares) but could retrieve a small piece of food without dropping it, which we termed a "retrieval," in most cases by gripping it with the dorsum of the thumb or palm (Fig. 4*B*, *Right*). Successful retrievals with the alternate grips persisted throughout the entire period of observation (91–132 d after the lesion) (Fig. 4 *A* and *B*, *Right*, and Fig. 4*C*, triangles). Thus, in both of these monkeys, continuous blockade of PNs from 1 to 2 wk before lesion resulted in minimum recovery of dexterous hand movements 3–4.5 mo after lesion (Fig. 4*C*), despite the intensive rehabilitative training required during the early period after the lesion (29). These findings contrasted obviously with those of previous studies, in which dexterous hand movements of all monkeys with C4–C5 CST lesions of this size largely recovered within 1–3 mo (15, 17, 21, 28). These findings suggested that the PNs played a key role in promoting the recovery of dexterous hand movements after the CST lesion.

Discussion

We showed that recovery of dexterous hand movements in monkeys after a CST lesion was perturbed following the blockade of synaptic transmission through the PNs in the midcervical cord segments. The effects of blocking PN transmission depended on when and how long it was blocked along with the ongoing stage recovery. Based on the present findings, we conclude that the PNs exert a stage-dependent contribution to recovery of hand dexterity in monkeys after CST lesions.

Previous studies in rodents suggested that PNs that bypass the lesion might mediate spontaneous recovery after spinal cord injury, which were confirmed by anterograde and retrograde labeling (32, 33) and by lesioning them with NMDA infusions (34). NMDA infusions cause hyperexcitability-induced death of cells that are located in injection sites. Transection of the corticospinal fibers at the rostral level to the PNs impaired recovery (35). These studies suggested a relationship of PNs to recovery, but such lesion might affect other group of neurons besides PNs, and demonstration of causal contribution of PNs was still indirect. Furthermore, because there are considerable differences in neural structures and body apparatus related to hand movements between rodents and primates (12, 36, 37), studies in nonhuman primates are critical for translating therapeutic strategies to treat spinal cord injury in humans (38, 39). Therefore,

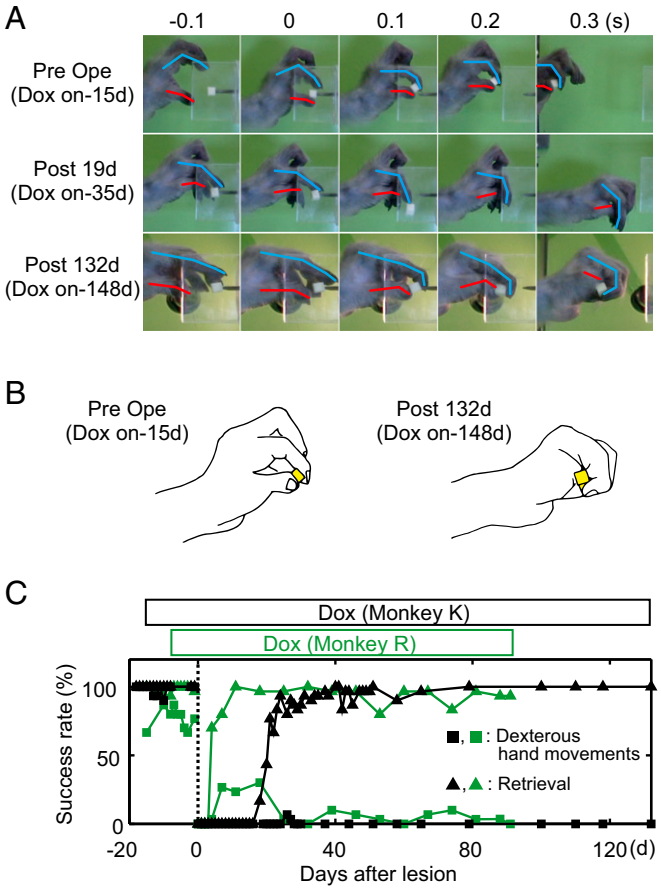

Fig. 4. Behavioral effects under continuous blockade of PNs after the CST lesion. (*A*) Recovery process of dexterous hand movements under continuous administration of Dox in monkey K. Upper row, before lesion (15 d after start of Dox). Middle row, 19 d after lesion (35 d after start of Dox). Bottom row, 132 d after lesion (148 d after start of Dox). Each panel is aligned to the moment of touching a piece of food. Blue line, the index finger; red line, the thumb. (*B*) Drawing of shapes of grasping before (15 d after start of Dox) and 132 d after lesion (148 d after start of Dox) in monkey K. (*C*) Recovery curves of success rates for dexterous hand movements (squares) and retrieval (triangles) under continuous administration of Dox (open boxes above the curves) after the lesion in monkey K (black lines) and monkey R (green lines). The data are aligned to the day of lesion (dashed line).

direct evidence to show the causal contribution of PNs to recovery in nonhuman primates was needed.

In the present transient blockade experiments, the effect of PN blockade on the success rate for dexterous hand movements after CST lesion was similar to that before lesion (Fig. 3*C*). If the PNs play a major role in recovery, their blockade should be expected to result in much severer impairment than in the intact state; however, the effects were weaker than expected. Two possible explanations might be considered for such low effectiveness of PN blockade on dexterous hand movements after lesion. First, it is possible that other descending pathways, such as the reticulospinal tract (20), the rubrospinal tract (14), and the remaining CST (18) or regenerated CST (5) contributed to the recovery. However, it is unlikely that either the reticulospinal or rubrospinal tracts contributed to recovery of dexterous hand movements in this experiment because synaptic transmission presumably through RSNs to the deep radial MNs was relatively minor (see "after C2 lesion" in Fig. 2*I* and Fig. S2), and descending axons of the rubrospinal tract were most likely transected in this animal model of spinal cord injury (40). Second, it is possible that the proportion of blocked neurons among the entire PN population was relatively small due to a limited rate of infection by the vectors.

In contrast, the effect of continuous blockade of PNs on recovery was strong and remarkable (Fig. 4). The extent of CST lesions in these two monkeys (K and R in Fig. 1*B*; 61.5% and 54.5%, respectively; Table S1) was not larger than those in previous studies (15, 17, 21, 28), which would lead us to expect near complete recovery. However, in these two monkeys, alternate grip strategies, which indicate insufficient recovery (41), persisted throughout the entire period of observation. Here, in contrast to the low effectiveness in the transient blockade experiments, recovery of dexterous hand movements was not achieved even 3–4.5 mo after the CST lesion (Fig. 4). Therefore, the context of PN blockade, its timing and length, appear to be critical for the extent of recovery. Blocking PN-mediated pathway at the same time of lesioning the CST made it more difficult to induce recovery than blocking it serially after lesioning the CST. Such phenomenon has also been previously explained by a changed function in unlesioned systems after serial spinal cord lesions in cats (42).

Our previous study showed that the training during the early period after lesion was critical for recovery (29). Early training might be effective in inducing the plasticity of the motor circuits; however, blockade of the PN-mediated pathway at this stage perturbed the effect of training, which might have resulted in poor recovery. Thus, the PN-mediated pathway was likely to have a more significant role in recovery of dexterous hand movements during the early period after lesion. In the present study, we intended to selectively target the descending branch of PNs, which can mediate cortical commands to the lower cervical MNs, using a double-virus infection method. However, it should be kept in mind that, when PN transmission was blocked by the Tet-on system, neural transmission through the ascending PN branches targeting the lateral reticular nucleus, one of the precerebellar nuclei, was also affected due to the bifurcating character of the PNs (23, 30). Therefore, the role of PNs in the recovery of skilled reach and grasp movements revealed in this study may also be dependent on their ascending pathways mediating the efference copy of movements (43), which will be a target of future studies.

As a methodological limitation of this study, we observed blocked neurons in the C5–C6 segments (Fig. 2*E* and Fig. S2), presumably caused by spread of viral vectors. We could not exclude completely the possibility for partial contribution of sINs in the C5–C6 segments to recovery after CST lesions. However, a few number of MNs in the C5 segment innervate digit muscles, which were thought to play a critical role in dexterous hand movements. Therefore, we did not consider that this affected the main conclusion of this study, namely, the contribution of PNs in the midcervical segments, which may partly include those in the C5 segment that are connected to digit MNs beyond segments, to recovery.

Recently, the reversible, pathway-selective blocking method combined with double-viral vectors was used also to investigate the recovery mechanism for skilled forelimb function in the stroke model rats (44, 45). Wahl et al. (44) showed that, after cortical lesions, administration of an antibody against the anti-neurite extension protein Nogo-A before intensive rehabilitative training enabled new and functional circuit formation of the CST fibers from the contralesional hemisphere to the contralesional half of the spinal cord, resulting in almost full recovery of skilled forelimb function. On the other hand, Ishida et al. (45) showed that the pathway from the motor cortex to the red nucleus contributed to recovery through intensive training after internal capsule hemorrhage. Thus, this method has great advantages in determining the role of a specific population of neurons in a recovery process after brain and spinal cord injury. Identification of a key neural element involved in the recovery mechanism is crucial, because it can promote development of novel treatments

combined with rehabilitation of motor disability following the brain/spinal cord injury. Selectively enhancing the plasticity of the pathways thus identified as being responsible for recovery will become a key technology to facilitate recovery in future studies.

Materials and Methods

The animal experimental procedures in this study were conducted in accordance with the principles of the National Institutes of Health and the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The protocols were approved by the Institutional Animal Care and Use Committee of the National Institutes of Natural Sciences. We recruited eight monkeys and then excluded two because the infection rate and/or expression of viral vectors in one monkey were judged to be obviously insufficient and the extent of lesion of the other monkey was too large; thus, the results of six monkeys are presented. The monkeys were first trained for a few weeks in a reach-and-grasp task described previously (15–17, 21–23) and in *Supporting Information* (*SI Materials and Methods*, *Behavioral Testing*, and Movie S1). The retrograde gene transfer vector (HiRet/FuG-E/NeuRet-TRE-EGFP.eTeNT) was then injected into the ventral horn of the spinal cord at C6–Th1 as previously described (23). One to 10 d after the injection, the anterograde vector (AAV-2/DJ-CMV-rtTAV16) was injected into the intermediate zone of the spinal cord at caudal C2 to caudal C4. Four monkeys (U, B, S, and N) received a total of four rounds of oral Dox (15 $mg \cdot kg^{-1} \cdot d^{-1}$, each round, 7–25 d) before and after the CST lesion. Two monkeys (K and R) received continuous administration of Dox from 8–16 d before the CST lesion to 3–4.5 mo after the lesion. Video recordings of reach and grasp movements before and after the lesion were analyzed off-line. The CST lesion was made by transecting the dorsolateral funiculus at C4–C5 under anesthesia with isoflurane (1–2%). After the end of behavioral observations, acute electrophysiological experiments were conducted on the cervical spinal cord in five of the six monkeys (data could not be obtained from monkey R due to a sudden change in the monkey's condition during the experiment) under anesthesia (see details in *SI Materials and Methods*). After transcardial perfusion with deep anesthesia, the brains and spinal cords were removed. Histological examinations were conducted with anti-GFP immunohistochemistry. Full materials and methods are described in *SI Materials and Methods*.

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