Spinal cord stimulation is a promising method for restoring motor function in paralyzed limbs following neurological damage to the descending pathways. Repetitive epidural or intraspinal stimulation produces functionally coordinated movements in patients (Harkema et al 2011, Gill et al 2018) and animals (Mushahwar and Horch 2000, Mushahwar et al 2000, Saigal et al 2004, Moritz et al 2007, Zimmermann et al 2011; Sunshine et al 2013, Kasten et al 2013), supporting the application of a neuroprosthetic device.

Stimulus outputs induced by subdural electrodes on the cervical spinal cord in monkeys

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Abstract

Objective. Spinal stimulation is a promising method for restoring the function of paralyzed limbs following neurological damage to descending pathways. The present study examined the forelimb movements and muscle responses evoked by subdural spinal stimulation of the cervical cord in sedated monkeys or during an arm-reaching task. Approach. We chronically implanted a platinum subdural electrode array with eight channels over the dorsal-lateral aspect of the cervical enlargement. The electrodes had a diameter of 1 mm and an inter-electrode center-to-center distance of 3 mm. Subdural spinal micro-stimulation was delivered at sites while the monkeys were sedated or performed arm-reaching movements. Main results. The evoked movements clearly showed the somatotopic map of the output sites; the electrodes located on the rostral cervical cord tended to induce movements of the proximal arm, whereas the caudal electrodes tended to induce movements of the distal joints, such as the wrist and digits. To document the muscle responses evoked by subdural spinal stimulation, stimulus-triggered averages of rectified electromyograms were compiled when the monkeys performed an arm-reaching task or were sedated. Under sedation, evoked facilitative muscle responses were observed in vicinity muscles. In contrast, during the task, stimulation evoked facilitative or suppressive responses in multiple muscles, including those located on proximal and distal joints, while somatotopy became blurred under sedation. Furthermore, stimulation during tasks activated synergistic muscle groups. For example, stimuli strongly facilitated finger extensor muscles, but suppressed the antagonist muscles. Significance. These dynamic changes in muscle representation by subdural cervical spinal stimulation between sedated and awake states help our understanding of the nature of spinal circuits and will facilitate the development of neuroprosthetic technology to regain motor function after neural damage to the descending pathways.

Introduction

Spinal cord stimulation is a promising method for restoring motor function in paralyzed limbs following neurological damage to the descending pathways. Repetitive epidural or intraspinal stimulation produces functionally coordinated movements in patients (Harkema et al 2011, Gill et al 2018) and animals (Mushahwar and Horch 2000, Mushahwar et al 2000, Saigal et al 2004, Moritz et al 2007, Zimmermann et al 2011; Sunshine et al 2013, Kasten et al 2013), supporting the application of a neuroprosthetic device...
for regaining lost motor function (Nishimura et al 2013a, Wagner et al 2018).

The output effects of spinal stimulation are well documented in anesthetized animals and in the spinalized condition in animals and humans. In anesthetized monkeys, cervical intraspinal microstimulation at a single or a few stimulus sites could produce synergistic reaching and grasping movements (Moritz et al 2007, Zimmermann et al 2011, Zimmermann and Jackson 2014). A previous study further demonstrated that intraspinal microstimulation could induce specific muscle responses with a clear rostro-caudal somatotopic representation in the rodent cervical spinal cord (Sunshine et al 2013). In the spinalized condition, repetitive epidural or intraspinal stimulation could induce stepping-like muscle activity in patients (Harkema et al 2011, Gill et al 2018, Wagner et al 2018) and animals with spinal cord injury (van den Brand et al 2012, Barthélemy et al 2007). Thus, the motor outputs elicited by spinal stimulation under sedation and spinalization are well reported, while they have not been documented in a systematic manner under voluntary movements.

In the present study, we chronically implanted a subdural array over the dorsal-lateral aspect of the cervical cord in monkeys. To demonstrate the motor effects of subdural spinal stimulation, we firstly investigated the rostro-caudal representation of both the motor outputs and muscle responses induced by stimulation under sedation. Then, we investigated them in conscious monkeys while they performed reaching, grasping, and retrieving tasks, to compare the muscle responses between states. As a result, we demonstrated that spinal stimulation using a subdural electrode array could drive multiple and functionally coordinated muscles and showed high specificity for muscle responses with a clear rostro-caudal representation of motor and muscle responses under sedation. In contrast, this somatotopic representation of muscle responses under sedation became blurred when the monkeys performed the tasks while awake, which may help our understanding of spinal circuits in the awake condition.

Materials and methods

The experiments were performed with two male Japanese macaque monkeys (2 Macaca fuscata: Monkey T; weight 7.1 kg, Monkey Y; weight 5.5 kg). All experimental procedures were performed in accordance with the guidelines of the National Institutes of Health and the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and were approved by the Institutional Animal Care and Use Committee of the National Institutes of Natural Sciences (Approval No. 14A125). The monkeys were monitored closely and animal welfare was assessed on a daily basis or, if necessary, several times a day.

Surgery

Both monkeys underwent surgery on different days to implant a subdural electrode array on the cervical cord and to implant electromyogram (EMG) wire electrodes in the upper limb muscles under anesthesia. However, in Monkey Y, a second operation for implantation of a subdural array was performed, since the implanted wires were disconnected at 1 month after the first implantation. All implant surgeries were performed using sterile techniques while the animal was anesthetized using 1%–2% isoflurane. We also continuously monitored electrocardiogram, pCO2, and arterial O2 levels. Dexamethasone, cephalexin, and ketoprofen were administered preoperatively and buprenorphine was given postoperatively.

Surgery for a subdural electrode array on the cervical cord

We chronically implanted a platinum subdural electrode array (Unique Medical Corporation, Tokyo, Japan) over the dorsal-lateral aspect of the cervical spinal cord, which had eight channels placed on the cervical enlargement (C5–T1) (figure 1). The subdural electrode array was implanted on bilateral sides in Monkey T and only on the right side in Monkey Y. The electrodes had a diameter of 1 mm and a center-to-center inter-electrode distance of 3 mm (figure 1(a)). A silver plate (3 × 2 mm) was used as a reference electrode. Laminectomy was performed on the C3 vertebra, and the lamina and dorsal spinal process of C3 were removed. An incision was made in the dura mater under the C3 vertebra. The subdural electrode array was slid into the subdural space from the incision site, and placed over the dorsal-lateral aspect of the cervical spinal cord, where the dorsal rootlets and corticospinal tract are located (figure 1(b)). The wires from the electrodes were routed into a silicone tube, which was glued with dental acrylic to bone screws placed in the lateral mass and C2 dorsal process, and routed to the monkey’s head to a connector. The dura was closed using 6.0 synthetic absorbable suture threads and covered with gel foam. The laminectomy was closed with acrylic cement, and a reference electrode was inserted into the supradural space between the dura and acrylic cement. The skin and underlying soft tissue were then sutured to close.

Surgery for EMG recording

EMG wires were surgically implanted in the right arm and hand muscles, which were identified by anatomical features and evoked movements by trains of low-intensity stimulation to the muscles. Bipolar, multi-stranded stainless-steel wires (Cooner Wire, Chatsworth, CA, USA) were sutured into each muscle, and the wires were routed subcutaneously to connectors (MCP-12; Omnetics, Minneapolis,
MN, USA) that were anchored to the skull. In Monkey T, the EMG electrodes were implanted in the following twelve muscles. One intrinsic hand muscle: first dorsal interosseous (FDI); three digit muscles: extensor digitorum 2 and 3 (ED23), flexor digitorum superficialis (FDS), and extensor digitorum communis (EDC); six wrist muscles: flexor carpi radialis (FCR), palmaris longus (PL), flexor carpi ulnaris (FCU), extensor carpi ulnaris (ECU), extensor carpi radialis (ECR), and brachioradialis (BR); and two elbow muscles: biceps brachii (BB) and triceps brachii (Triceps). In Monkey Y, the EMG electrodes were implanted in the following fifteen muscles. Two intrinsic hand muscles: adductor pollicis (ADP) and abductor pollicis longus (APL); three digit muscles: FDS, flexor digitorum profundus (FDP), and extensor digitorum 4 and 5 (ED45); seven wrist muscles: FCR, PL, FCU, ECU, ECR, BR, and pronator teres (PT); two elbow muscles: BB and Triceps; and one shoulder muscle: deltoid.

Experimental setup

In the experiments with subdural spinal stimulation administered under sedation, the monkeys were seated quietly in a primate chair with their head fixed in a frame attached to the chair (figure 2(a)), and then sedated with ketamine (10 mg kg\(^{-1}\), i.m.). Additional doses of ketamine were given as needed to eliminate spontaneous movements during the recording sessions.

In the experiments performed in the awake state, subdural spinal stimulation was administered when the monkeys performed a task in which they reached, grasped, and retrieved a small piece of sweet potato (~7 × 7 × 7 mm) while sitting on a monkey chair. The food piece was positioned at the height of the monkey’s shoulder, so that muscle activity of the shoulder, elbow, wrist, and hand was observed during the task (figures 2(e) and (f)).

Stimulus protocol

In the experiments under sedation (figures 2(a)–(d)), subdural spinal stimuli consisting of three current, biphasic square-wave pulses of 200 Hz with 0.2 ms duration were delivered through a single electrode. Although most studies of intra/surface cortical or spinal stimulation in sedated animals have used longer stimulus trains, we chose these brief stimulus trains to minimize the amount of additional activity generated by the temporal summation of the postsynaptic effect and the current required to evoke a motor response in sedated animals (Moritz et al 2007). Each stimulus train was delivered with an interval of 1000 ms. Evoked movements and muscle twitches induced by subdural microstimulation on the spinal cord were observed carefully as stimulus intensity (50–1000 µA) was increased gradually. The evoked joint movements and muscle twitches were detected by visual inspection, and further monitored by direct muscle palpation. The minimum stimulus current necessary to evoke joint movements or muscle twitches was defined as the motor threshold (MT) for each electrode. After detecting the MT at each stimulus site, the motor outputs were investigated using the stimulus-triggered average (StTA) of rectified EMGs at stimulus currents of MT × 1.0, MT × 1.2, and MT × 1.5 at each stimulus site. Each stimulus train was delivered with an interval of 260 ms for compiling the StTA.

In the experiments in the awake state (figures 2(e)–(h)), one pulse of a biphasic square-wave with a duration of 0.2 ms and an interval of 122 ms was applied through a single electrode during the task. Since the excitability of motoneurons becomes high during the
task, the postsynaptic effects on motoneurons will be more sensitive compared with animals in the sedated state. Therefore, we chose a single pulse to minimize the temporal summation of the postsynaptic effect on motoneurons. At each stimulus site, we performed subdural spinal stimulation with different stimulus currents of 250, 300, 350, and 400 µA in Monkey T and 400, 450, 500, 550, and 600 µA in Monkey Y.

Data collection

During the experiments, the trigger pulses of stimulation and EMG activity recorded from the implanted EMG wires were recorded simultaneously at a sampling rate of 3.5 kHz through a 1401 A–D converter (Cambridge Electronic Design, Cambridge, UK) onto a computer using Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Data analysis

The StTA technique used in the present study was developed by Cheney and Fetz (1985) and is described fully in the work of Cheney et al (1991). The averages of EMG data were compiled over a 60 ms period (20 ms before the trigger to 40 ms after it). The assessment of muscle responses induced by subdural spinal stimulation under sedation was based on the StTA of at least 50 trigger events. However, to avoid contamination of stimulus artifacts with the muscle responses, muscle activity recorded during three stimulus phases of 0–0.2 ms, 5.0–5.2 ms, and 10–10.2 ms was removed and
performed in each monkey. Cervical subdural stimulation at the current of the over the C5–T1 region of the cervical spinal cord. Implanted subdural platinum electrode array by subdural spinal stimulation via a chronically stimulation under sedation Evoked muscle responses by subdural spinal stimulation most commonly evoked movements of the digits (n = 10), but also produced wrist (n = 4), elbow (n = 6), shoulder (n = 2), and trunk (n = 3) movements. Moreover, the electrodes located on the rostral cervical cord (i.e. Elec. Nos. 1–3) tended to induce movements in the proximal joints, such as the trunk, shoulder, or arm, while caudal ones (Nos. 4–7) specifically induced distal movements of the wrist and digits. Overall, the clear somatotopic arrangement of outputs was generally observed under sedation (figure 3(A)).

Currents of subdural spinal stimulation higher than the MT often evoked movements in multiple joints of the upper limb. In detail, at currents of MT × 1.2 and ×1.5, subdural spinal stimulation induced at least two discrete movements at 50.0% and 82.1% of the stimulus sites, respectively. The extent of the output sites sometimes reached the lower extremities ipsilateral to the stimulation site including digit of foot, ankle, and knee joints (i.e. Elec. Nos. 2 and 3 in figure 3(A)d). At the vast majority of stimulus sites (89%) for all stimulus currents, subdural spinal stimulation activated forelimb movements ipsilateral to the stimulus sites, while the remaining 11% evoked contralateral movements, especially in Monkey Y. The stimulus current threshold needed to evoke a movement was lower than 500 µA across the electrodes in both monkeys, and was distributed nearly uniformly over the output sites (p = 0.42, one-way ANOVA, figure 3(B)), suggesting that subdural spinal stimulation could equally drive differential forelimb movements with a relatively low stimulus current.

To investigate the receptive fields of the muscle responses induced by subdural spinal stimulation, defined as the ‘muscle field’, the PSTEs of the recorded forelimb EMG activity were evaluated by compiling the StTAs of rectified EMGs under sedation in both monkeys. The StTAs of representative muscles and electrode sites sometimes reached the lower extremities ipsilateral to the stimulation site including digit of foot, ankle, and knee joints (i.e. Elec. Nos. 2 and 3 in figure 3(A)d). At the vast majority of stimulus sites (89%) for all stimulus currents, subdural spinal stimulation activated forelimb movements ipsilateral to the stimulus sites, while the remaining 11% evoked contralateral movements, especially in Monkey Y. The stimulus current threshold needed to evoke a movement was lower than 500 µA across the electrodes in both monkeys, and was distributed nearly uniformly over the output sites (p = 0.42, one-way ANOVA, figure 3(B)), suggesting that subdural spinal stimulation could equally drive differential forelimb movements with a relatively low stimulus current.

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To determine statistically significant differences in the MT of evoked movements in the trunk, shoulder, elbow, wrist, and digits, one-way factorial analysis of variance (ANOVA) with Bonferroni’s correction for post hoc multiple comparisons was performed (figure 3(B)). To determine statistically significant differences in the onset of muscle responses between stimulus currents in the seated (figure 4(D)) and awake (figure 6(C)) states, one-way factorial ANOVA with Bonferroni’s correction for post hoc multiple comparisons was performed in each monkey. To determine statistically significant differences in the onset of muscle responses between the seated and awake states (figure 8(B)), an unpaired t-test was performed in each monkey.

Results

Evoked muscle responses by subdural spinal stimulation under sedation

We investigated the motor responses induced by subdural spinal stimulation via a chronically implanted subdural platinum electrode array over the C5–T1 region of the cervical spinal cord. Cervical subdural stimulation at the current of the
Rostro-caudal mapping of peak SD showed that subdural spinal stimulation using the rostral electrodes (i.e. Elec. Nos. 1 and 2) activated the elbow muscles, such as BB (figure 4(B)). Subdural spinal stimulation using more caudal electrodes (i.e. Elec. Nos. 4–6) mostly activated the wrist muscles, such as the BR, ECU, EDC, ECR, and FCR (figure 4(B)). In the most caudal electrodes (i.e. Elec. Nos. 6 and 7), subdural spinal stimulation strongly activated digit muscles, such as the FDS, ED23, and FDI (figure 4(B)). These results indicated that the somatotopic arrangement of PSTEs was observed clearly, which corresponded to that of the evoked movements. Moreover, increasing the stimulus current facilitated the activation of multiple muscles and produced higher PSTEs in the recorded muscles (compare among figures 4(B(a)–(c)). Thus, the percentage of muscles that showed significant PSTEs was increased in proportion to stimulus current in both monkeys (figure 4(C)). In contrast, the onset latency of muscle responses was not different.
Figure 4. Stimulus outputs to forelimb muscles under sedation. (A) The STAs of seven recorded muscles over four electrodes at a stimulus current of $MT \times 1.2$ were compiled under sedation in Monkey T. The numbers on the left side indicate electrode number. The gray vertical line in each STA shows stimulus timing. The two horizontal lines represent $\pm 2$ SDs of the magnitude of STAs calculated during the baseline period. Subdural spinal stimulation on the electrode located on the rostral cervical cord (Elec. No. 1) evoked the BB, whereas caudal ones (Elec. Nos. 4–7) evoked multiple wrist and digit muscles, including the ECU, FCR, FCU, EDC, FDS, and FDI, showing the clear somatotopic representation at the stimulus sites under sedation. (B) Mapping of the magnitude of PSEs by subdural spinal stimulation at currents of $MT \times 1.0$ (a), $MT \times 1.2$ (b), and $MT \times 1.5$ (c) in all of the recorded muscles and electrodes. The proximal muscles are located on the left side, while the distal muscles are located on the right side. Increasing the stimulus current facilitated the activation of multiple muscles and produced higher PSEs in each muscle. Rostral electrodes tended to facilitate arm muscles, whereas caudal ones tended to facilitate digit muscles, suggesting that PSE mapping showed the clear somatotopic representation. (C) Distribution of the percentage of muscles showing significant PSEs in black (non–significant PSEs are shown in white). The percentage of activated muscles increased in proportion to the strength of the stimulus current. (D) Effect of current intensity of $MT \times 1.0$, $MT \times 1.2$, and $MT \times 1.5$ on the onset latency of muscle responses in Monkeys T (a) and Y (b) during sedation. An asterisk indicates significant difference ($p < 0.05$, one–way factorial ANOVA with Bonferroni’s correction for post hoc multiple comparisons).
among stimulus currents in Monkey T (figure 4(D(a)), \( p = 0.55 \), one-way ANOVA), while the onset latency for MT \( \times 1.5 \) were significantly shorter than those for MT \( \times 1.0 \) in Monkey Y (figure 4(D(b)), \( p < 0.05 \), one-way ANOVA with Bonferroni’s correction for post hoc multiple comparisons).

Evoked muscle responses by subdural spinal stimulation in the awake state

We next examined how the observed somatotopic arrangement of the muscle fields induced by subdural spinal stimulation under sedation was modulated when the monkeys actively performed a task. To characterize this, we investigated PSTEs by subdural spinal stimulation when the monkeys performed an arm-reaching task. As a result, subdural spinal stimulation typically evoked facilitative and suppressive effects in multiple muscles simultaneously. In some representative profiles of the PSTEs (figure 5(A)), subdural spinal stimulation strongly facilitated extensor muscles (e.g. ECR, EDC, and ECU) and simultaneously suppressed antagonist flexor muscles (e.g. FCU, FDS, and FCR), suggesting that the facilitative and suppressive effects were organized by functionally segregated synergistic muscle groups. Conversely, other PSTE profiles showed only a facilitative effect in all of the recorded muscles (figure 5(B)). However, there was a tendency that the magnitude of PSTEs was stronger in extensor muscles and weaker in flexor muscles (figure 5(B)).

The observed post-stimulus facilitative and suppressive effects were modulated differentially depending on the stimulus current (figure 6(A)). For example, the post-stimulus facilitative effect became stronger in proportion to the stimulus current (figures 6(A(a) and (c)). In another case, as the post-stimulus facilitative effect became stronger, the onset of the PSTE became faster in proportion to the stimulus current (figure 6(A(c))). In some PSTE profiles, both the post-stimulus facilitative and suppressive effects were increased simultaneously as the stimulus current increased (figure 6(A(d))). Taken together, as the stimulus current increased, the post-stimulus facilitative and suppressive effects were intricately changed depending on the recorded muscles and stimulus sites.

To characterize further the details of PSTEs during active movements, we compiled the MPC of each StTA profile in each recorded muscle, stimulus site, and stimulus current in Monkey T (figure 6(B)). Post-stimulus facilitative and suppressive effects were differently observed depending on the stimulus site. Among the stimulus sites and recorded muscles, subdural spinal stimulation induced mostly post-stimulus facilitative effects (39.0%, 42.9%, and 55.8% at 300, 350, and 400 \( \mu \text{A} \), respectively), but also suppressive effects (9.1%, 7.8%, and 10.4% at 300, 350, and 400 \( \mu \text{A} \), respectively). Interestingly, at more than half of the total stimulus sites, suppression could simultaneously drive post-stimulus facilitative and suppressive effects in multiple muscles (71.3%, 71.3%, and 57.1% stimulus sites at currents of 300, 350, and 400 \( \mu \text{A} \), respectively).
Figure 6. Effect of current intensity on stimulus outputs to forelimb muscles during voluntary movements. (A) PStEs by subdural spinal stimulation at various stimulus currents in representative stimulus sites and muscles. (a) Increase in the post-stimulus facilitative effect in proportion to stimulus current in the ECR by subdural spinal stimulation through Electrode No. 7. (b) Increase in both the post-stimulus facilitative and inhibitory effects in the Triceps by subdural spinal stimulation through Electrode No. 1. (c) The onset of PStE was faster as the amount of post-stimulus facilitation was increased in the ECR by subdural spinal stimulation through Electrode No. 5. (d) Increase in the post-stimulus facilitative effect and decrease in the inhibitory effect in proportion to stimulus current in the ECU by subdural spinal stimulation through Electrode No. 5. (B) Mapping of MPC of PStEs was performed in each muscle. Red indicates post-stimulus facilitative effects, and blue indicates post-stimulus suppressive effects. Typically, post-stimulus facilitative and suppressive effects were observed simultaneously in multiple muscles by subdural spinal stimulation on a single electrode. However, subdural spinal stimulation on Electrode Nos. 2 and 4 evoked only post-stimulus facilitative effect across the muscles. The post-stimulus suppressive effect was observed specifically in the FCU and FCR. In general, little somatotopic representation at the stimulus sites was confirmed. C: Effect of current intensity on the latency of muscle responses in Monkeys T (a) and Y (b) during the awake state.
respectively). Most of the post-stimulus suppressive effects were observed in the Triceps, FCR, and FCU. However, the rostro-caudal somatotopic representation of the MPC profiles was not observed clearly, indicating that the somatotopic arrangement of PSTEs was organized differentially between the sedated and awake states. The onset latency times of muscle responses during the task were not different among stimulus currents in both monkeys, as observed under sedation (figure 6(C), Monkey T, \( p = 0.98 \); Monkey Y, \( p = 0.16 \); one-way ANOVA with Bonferroni’s correction for post hoc multiple comparisons).

Mapping of the MPC induced by subdural spinal stimulation showed either the post-stimulus facilitative or suppressive effects in each recorded muscle. However, it is important to note that subdural spinal stimulation sometimes induced both the post-stimulus facilitative and suppressive effects in a single muscle (figure 6(A(b))). Therefore, to characterize the details of the types of PSTEs, we sorted the PSTE profiles shown in figure 7(A) by the formation of facilitative and suppressive effects as follows: ‘facilitation only’ (figure 7(A(a))), ‘facilitation + suppression’ (figure 7(A(b))), ‘facilitation + suppression + facilitation’ (figure 7(A(c))), ‘suppression only’ (figure 7(A(d))), ‘suppression + facilitation’ (figure 7(A(e))), and ‘other’ types (figure 7(A(f))). As a result, we found that pure post-stimulus facilitation was mostly common at every stimulus current in both monkeys. However, as well as the pure facilitative effect, the other types were evident among the recorded sites. In Monkey Y, the ‘other’ type was increased as the stimulus current was increased.

| A | B |
|---|---|
| ![Figure 7. Variety of stimulus outputs to forelimb muscles during voluntary movements. A: Sorting the PSTEs by the formation of facilitative (F) and suppressive (S) effects. (a) F only; (b) F + S; (c) F + S + F; (d) S only; (e) S + F; (f) F + S + F. B: The distribution of PSTE types at stimulus currents of 250–400 µA in Monkey T (a) and 400–600 µA in Monkey Y (b). Pure post-stimulus facilitation was most common at every stimulus current in both monkeys. As well as the pure facilitative effect, the other types were evident among the recorded sites. In Monkey Y, the ‘other’ type was increased as the stimulus current was increased.](https://example.com/f7.png) | ![Figure 7.](https://example.com/f7.png) |

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**Figure 7.** Variety of stimulus outputs to forelimb muscles during voluntary movements. A: Sorting the PSTEs by the formation of facilitative (F) and suppressive (S) effects. (a) F only; (b) F + S; (c) F + S + F; (d) S only; (e) S + F; (f) F + S + F. B: The distribution of PSTE types at stimulus currents of 250–400 µA in Monkey T (a) and 400–600 µA in Monkey Y (b). Pure post-stimulus facilitation was most common at every stimulus current in both monkeys. As well as the pure facilitative effect, the other types were evident among the recorded sites. In Monkey Y, the ‘other’ type was increased as the stimulus current was increased.
Comparison of the onset latency of muscle responses between the sedated and awake states

To examine differences in onset latency between the states, we compared the onset latency of muscle responses to subdural spinal stimulation between animals under sedation and during the awake state. Figure 8(A) shows examples of muscle responses in the sedated and awake states at the highest current in each state. The onset latency of muscle responses in the awake state were a few milliseconds faster than those under sedation. This tendency was further evidenced by population analysis (figure 8(B)). The onset latencies of muscle responses in both monkeys were significantly faster in the awake state than under sedation ($p < 0.001$, unpaired $t$-test).

Discussion

In the present study, we investigated the motor and muscle effects of subdural cervical spinal cord stimulation during sedated and awake states in monkeys. Subdural spinal stimulation over the C5–T1 region could drive a variety of functionally segregated synergistic muscle responses under sedation, showing the clear somatotopic rostro-caudal organization of the output sites. However, this somatotopy became blurred when the monkeys were awake, and instead, simultaneous responses of post-stimulus facilitative and suppressive effects were generally observed following subdural spinal stimulation at multiple stimulus sites. Moreover, the patterns of the facilitative and suppressive effects were often intermixed and modulated differentially depending on the stimulus current. These dynamic changes in muscle representation by subdural spinal stimulation between sedated and awake states may help our understanding of the nature of spinal circuits in the awake condition and facilitate the development of potential applications of subdural spinal stimulation in patients with damage to descending pathways.

Somatotopic organization of motor outputs under sedation

Under sedation, we found the clear rostro-caudal somatotopic representation of the evoked movements at MT by subdural spinal stimulation (figures 3(A) and 4(B)). The movements evoked by subdural spinal stimulation over C5–T1 corresponded approximately to the innervated muscles of the motor nuclei and fibers of a single nerve root over C5–T1, suggesting that subdural spinal stimulation under sedation can drive selective motoneuron pools and/or ventral roots according to the rostro-caudal order of the innervated muscles. However, the onset latency of muscle activation under sedate state was slower than that during awake state (figure 8(B)), indicating that direct activation of ventral roots and motoneuron under sedate state is unlikely. The result of intraspinal stimulation in dorsal aspect of cat lumbar cord is suggested to activate afferent fibers first at lower intensities than needed to activate the cell bodies or axons of motor neurons (Gaunt et al 2006). The subdural arrays used in our study covered the dorsal-lateral aspect of the cervical spinal cord beneath the dorsal root and dorsolateral funiculus, which contains corticospinal and rubrospinal tracts. From these previous and current findings, it is plausible to postulate that subdural spinal stimulation would firstly drive the afferent fibers adjacent to the stimulus electrodes, and then excite local motor neurons trans-synaptically by activating a sufficient number of their inputs within the segmental spinal circuitry. As stimulus current increased, currents drove the commissural interneurons activating the limb contralateral to the stimulation site (figure 3(A)) and the intersegmental segmental spinal circuitry evoked movements of multiple joints in the upper limb. Furthermore, the extent of the output sites at higher current stimulation at rostral electrodes also activated the lower extremities ipsilateral to the stimulation site (figure 3(A(d))), indicating that subdural stimulation at a higher current activated the pathways of interlimb reflexes originating cutaneous afferents via the long propriospinal neurons innervating the lumbar enlargement (Miller et al 1977, Haridas et al 2003, Hurteau et al 2018). In addition, stimulation might activate descending tracts such as the corticospinal, rubrospinal, reticulospinal tracts directly those innervating the lumbar enlargement. The predominance of evoked movements in digits following subdural spinal stimulation was also observed (figure 3), similar to a previous study of cervical intraspinal stimulation in monkeys (Moritz et al 2007). This predominance may be expected based on the increased number of corticomotoneuronal projections to the spinal motoneurons of digit muscles in primates (Cheney and Fetz 1985, Lawrence et al 1985). Altogether, subdural spinal stimulation under sedation likely drives spinal motoneurons indirectly by activating a sufficient number of input fibers onto motoneurons.

Dynamical modulation of muscle fields between the sedated and awake states

Our results also showed that the clear rostro-caudal somatotopic representation of output effects under sedation became blurred when the monkeys were awake and performing a task (compare figures 4(B) and 6(B)). This differential pattern of output effects between these states was similarly discussed in previous studies of lumbar intraspinal stimulation (Mushahwar et al 1998, 2002, 2004, Lemay and Grill 2004). Mushahwar et al (2002) showed that flexion and extension movements evoked by lumbar intraspinal stimulation appeared equally under anesthesia; however, they were dominated by flexion movements after decerebration and spinalization.
This indicates that muscle outputs induced by intraspinal stimulation are influenced by the activity level of the inputs and the state of the spinal cord. It can also be speculated that when the monkeys were performing the reaching, grasping, and retrieving tasks, the activity levels in many types of fibers, such as peripheral, descending tracts, and intraspinal neurons, were changed. It has been suggested that the activation of afferent projections influences motoneurons located up to several segments from the stimulation site (Gaunt et al 2006); therefore, the responses mediated by dorsal roots may be less dependent on the location of the stimulating electrodes. In addition, a previous study of cervical intraspinal stimulation in monkeys demonstrated similar findings of the lack of a somatotopic organization of upper-limb movements under sedation (Moritz et al 2007). They discussed the role of propriospinal neurons that projected across segments in mediating the effects of intraspinal stimulation. It is a well-known fact that the indirect pathway via C3–C4 propriospinal neurons as well as the direct corticomotoneuronal pathway can convey the commands for dexterous movements, such as precision grip (Isa et al 2007, Alstermark and Isa 2012). Therefore, the changes in the activation levels of these afferent and propriospinal neurons may help to explain the lack of somatotopic rostro-caudal representation of the output sites in the awake state.

We also found that the onset latency of muscle responses in the behaving state were faster than that under sedation (Figure 8). This difference might reflect the excitability of spinal circuits and the pathways of muscle responses. Under sedation, the excitability of spinal circuits might be substantially lower than in the behaving state and requires the temporal summation of injection currents to exceed the firing threshold of spinal motoneurons, resulting in slower onset latency. In contrast, the excitability of spinal circuits in the behaving state might be higher, implying subdural spinal stimulation could activate the minimum number of pathways such as Ia fibers that drive the spinal reflex pathway or motor axons directly.

**Facilitative and suppressive muscle responses under the behaving state**

Another important finding of the present study was that among more than half of the total stimulus sites, subdural spinal stimulation could simultaneously drive post-stimulus facilitative and suppressive effects in multiple muscles (figure 6(B)), which was not observed under sedation. In some cases, the distribution of the post-stimulus facilitative and suppressive effects was divided between extensors and flexors (figure 5(A)). This result was consistent with a previous study using intraspinal stimulation to show facilitative or suppressive effects in multiple muscles in an awake monkey with a spinal cord injury (Nishimura et al 2013a). The post-stimulus suppressive effects were probably mediated by, at least, a disynaptic link via inhibitory interneurons, while the facilitative effects were via excitatory interneurons. Moreover, the higher selectivity between flexors and extensors seen in the present study may reflect the functional organization of Ia or cutaneous reflex circuits, in which afferent axons directly excite synergistic muscles and inhibit antagonistic muscles via inhibitory spinal interneurons (Perlmutter et al 1998, Illert and Kümmel 1999, Nishimura et al 2013a). We also found that in some stimulus sites and currents, only the facilitative effect was induced in the recorded forelimb muscles (figure 5(B)), implying that, in some cases, subdural spinal stimulation could activate motor axons directly or specifically drive excitatory interneurons without the mediation of inhibitory interneurons.

The observed post-stimulus facilitative and suppressive effects in awake monkeys were changed dynamically depending on the stimulus current (figure 6(A)), indicating that the recruitment of neural elements increased as the stimulus current increased. Overall, the muscle effects observed under the behaving state, which were generally intermixed with post-stimulus facilitative and suppressive effects depending on the stimulus sites and currents, indicate the complex nature of spinal circuits. These selective facilitative or suppressive effects by subdural spinal stimulation may lead to more natural and coordinated movements, which were close to the situation of voluntary joint movements in natural conditions.

**Toward clinical application**

Spinal cord stimulation provides a promising target for neuroprosthetics to restore impaired functionally coordinated movements in upper (Moritz et al 2007, Zimmermann et al 2011, Nishimura et al 2013b, Zimmermann 2014, Sharpe and Jackson 2014) or lower limbs (Mushahwar and Horch 1998, Minassian et al 2004, Sasada et al 2014, Wagner et al 2018) after neural damage to descending pathways. In general, the advantage of spinal cord stimulation would be the naturalistic recruitment of multiple muscles through the activation of the surviving spinal circuitry and the small number of stimulation channels required to produce functionally segregated synergistic muscle activation (Bamford et al 2005, Jackson and Zimmermann 2012, Mondello et al 2014). An invasive approach using intraspinal stimulation has also been demonstrated to restore functional wrist movements after spinal cord injury or inactivation of the primary motor cortex in primates (Nishimura et al 2013b, Zimmermann 2014). Moreover, Nishimura et al (2013a) succeeded in strengthening corticospinal connections by a Hebbian process using brain-controlled intraspinal stimulation, suggesting that spinal stimulation may be suited for chronic use during the rehabilitation of patients with incomplete injuries by strengthening the surviving descending.
Figure 8. Comparison of the onset latency of muscle responses between the sedated and awake states. (A) Examples of the onset latency of muscle responses in the sedated (a) and awake (b) states. (a) StTAs of rectified EMGs to 50 stimuli at a stimulus current of MT \times 1.2 (432 \mu A). The horizontal dashed gray lines represent \pm 5 SDs of the magnitude of StTAs calculated during the baseline period (the interval from 50 to 5 ms preceding the stimulus trigger pulse). (b) StTAs of rectified EMGs to 150 stimuli at 300 \mu A. The two horizontal lines represent \pm 5 SDs of the magnitude of StTAs calculated during the baseline period. Data obtained from Monkey T. The arrows represent the onset latency in each muscle. (B) Population data of the onset latency of muscle responses between the sedated and awake states in Monkeys T (a) and Y (b). The error bars indicate SD. An asterisk indicates a significant difference (p < 0.001, unpaired t-test).
pathways. However, this invasive approach also has some technical limitations such as tissue damage by the surgical implant (Biran et al 2005, Bamford et al 2010) and the deterioration of stimulus outputs by electrode capsulation (Grill et al 2009, Bamford et al 2010). The long-term stability of the muscle effects of subdural approaches should be investigated in a future study.

Another important limitation of this study is that the results were obtained using intact animals, which are difficult to compare directly to animals with brain or spinal cord damage. The plastic changes to spinal cord circuitry following injury may alter the responses to stimulation. Nevertheless, a recent study in rodents reported comparable responses to intraspinal stimulation in healthy and chronic spinal cord injured animals (Sunshine et al 2013). Additionally, in a monkey with a partial injury at the upper cervical cord, it was reported that intraspinal cervical stimulation caudal to the lesion site could similarly evoke facilitative and suppressive effects in multiple muscles (Nishimura et al 2013b). However, further systematic investigations are needed to document the reorganization of spinal circuits and residual spinal function in animals after spinal cord injury.

Conclusion

The present study showed the motor and muscle effects of subdural spinal stimulation during sedated and awake states in monkeys. Under sedation, subdural spinal micro-stimulation over the C5–T1 region could induce the activation of functionally segregated muscle groups and show the clear somatotopic rostro-caudal organization of the output sites. When the monkeys were awake, however, this somatotopy became blurred, and the simultaneous post-stimulus facilitative and suppressive effects were generally observed. Typically, spinal stimulation evoked facilitative or suppressive effects in multiple muscles, including those located on proximal and distal joints. Furthermore, stimulation activated synergistic muscle groups. For example, stimuli facilitated movements of finger extensor muscles, but suppressed the antagonist muscles. These stimulus effects modulated depending on the stimulus currents. These dynamic changes in muscle representation by subdural spinal microstimulation between sedated and awake states may help to understand the nature of spinal circuitry and facilitate the development of potential neuroprosthetic applications in patients with damage to descending pathways.

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Competing financial interests

The authors declare no competing financial interests.

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