Functional role of the mesolimbic system in motor control

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Summary

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General introduction

It is believed that higher motivation boosts motor performance in healthy population and/or functional recovery after neuronal damage in patients. However, the neural mechanism underlying such psychological effects on motor outputs or recovery is still unclear. Several recent studies (Pessiglione *et al*., 2007; Thabit *et al*., 2011; Schmidt *et al*., 2012; Marsh *et al.*, 2015) imply that brain areas related to regulation of motivation modulate the brain activity of motor-related areas, which would facilitate motor outputs. The mesolimbic system consisting of the ventral tegmental area, the medial part of substantia nigra pars compacta in the ventral midbrain (VM) and the ventral striatum (VSt) are considered to process motivation for goal-directed behaviors (Salamone and Correa, 2012; Floresco, 2015).

However, it is still unclear whether the mesolimbic system modulates the activity of motor-related areas including M1 and facilitate the motor outputs and functional recovery. To address this question, in the Part I, I investigated the polysynaptic anatomical and functional connection from the VM to the spinal circuits controlling muscle activities in healthy monkeys. Furthermore, in the Part II, I investigated the functional significance of the VSt in the control of dexterous and less dexterous finger movements and the effect of the permanent lesion of the VSt on motor recovery of dexterous and less dexterous finger movements after SCI.

Part I

Title: Macaque ventral midbrain facilitates the output to forelimb muscles via the primary motor cortex

Introduction

It is believed that higher motivation boosts motor performance especially in prestigious competitions in sports. Several human studies (Pessiglione *et al*., 2007; Thabit *et al*., 2011; Schmidt *et al*., 2012) and a monkey study (Marsh *et al.*, 2015) have demonstrated that the incentive motivation modulates the activity of the primary motor cortex (M1). Thus, the M1 activity could be under control of incentive motivation. Reward/motivation-related activities are generally considered to be associated with activation of the ventral midbrain (VM), such as the ventral tegmental area (VTA) and substantia nigra (SN) pars compacta (Schultz *et al*., 1997, 1998; Matsumoto and Hikosaka, 2009). It has been repeatedly reported that the VM containing the VTA, SN and retrorubral field (RRF) has widespread direct projections to the frontal cortical areas including the M1 in non-human primates (Lewis *at al.*, 1987; Gasper *et al*., 1992; Williams and Goldman-Rakic, 1998). Thus, the VM might exert an impact on motor behavior through modulating the M1 activity. However, despite the evidence for the anatomical linkage from the VM to the M1, whether and how such a projection is effective to modulate the motor outputs remains elusive.

To address this issue, I first investigated the existence of multisynaptic projections from the VM to the spinal cord by injecting rabies virus that allows retrograde transsynaptic transport into the cervical enlargement. Secondarily, I investigated the functional significance of the identified VM–spinal pathway in modulating the motor output. Finally, I investigated the causal contribution of the M1 to the muscle responses induced by the VM stimulation.

Methods

***Subjects*:** Five monkeys were used in the present study. I performed two kinds of experiments, the neuroanatomical and electrophysiological experiments. Two adult macaque monkeys (*Macaca mulatta*; Monkey F, female, 5.0 kg; Monkey L, female, 5.0 kg) were used for the neuroanatomical experiments. Three adult macaque monkeys (*Macaca mulatta*; Monkey T, male, 8.0 kg, *Macaca fuscata*; Monkey D, female, 5.5 kg; Monkey Y, female, 6.5kg) were used for a series of electrophysiological experiments.

Part 1. Neuroanatomical experiment

***Rabies virus injections*:** Rabies virus that permits retrograde transsynaptic transport was injected into the cervical enlargement of the spinal cord in macaque monkeys. Thirteen penetrations spaced 2 mm apart were made rostrocaudally from C6 to T1 spinal segment. Small amounts of the virus were injected at intermediate zone and ventral horn, targeting around layer VII and IX where the spinal premotor interneurons (Perlmutter *et al.*, 1998) and motoneurons (Jenny and Inukai, 1983; Chiken *et al*, 2001) are distributed, respectively (0.5-0.6µl x 2 site per track). A total of 13 (Monkey F) or 15.6 µl (Monkey L) of the viral suspension was injected into the spinal cord. Survival time for virus was set at 3.5 (Monkey F) or 3.75 days (Monkey L). Neuronal labeling was plotted on tracing of equidistant coronal sections (500 µm) through the VM.

Part 2. Electrophysiological experiment

To clarify the functional connectivity between the VM and muscles, two kinds

of electrophysiological experiments were performed. One was recording cortical responses and muscle responses induced by the VM stimulation (Monkey D and T). Another was recording muscle responses before and during the M1 inactivation (Monkey D and Y).

***Surgery*:** All three monkeys (Monkey T, D, Y) were chronically implanted a chamber for the VM stimulation and micro-wires into upper limb muscles for electromyogram (EMG) recording. Monkey D and Monkey T were also chronically implanted electrocorticogram (ECoG) arrays for recording cortical responses from frontal cortical areas including the lateral prefrontal cortex (LPFC), orbitofrontal cortex (OFC) and sensorimotor cortex such as the premotor area (PM) and M1. In addition, Monkey D and Monkey Y were implanted an additional chamber for M1 inactivation study.

***VM stimulation*:** To record evoked responses from cortical areas and muscles by VM stimulation, stimuli were delivered using bipolar electrodes (impedance: 500-600 kΩ) under sedation with ketamine (10 mg/kg, i.m.). Stimulus trains with current of 100, 300 or 500 μA were delivered to the target location. Stimuli consisted of 3 biphasic pulses with 0.2 ms square-wave duration at a frequency of 300 Hz. For recorded data, stimulus-triggered averages of ECoG and rectified EMG were performed. The StTAs of ECoG responses were identified as having significant responses when the evoked response exceeded ± 2 SD of the baseline (-100 to 0 ms) between 0 and 200 ms after stimulus onset. Each muscle was considered to have a significant activation when the magnitude of StTA of the rectified EMG response exceeded + 2 SD of the baseline and had a total duration ≥ 3 ms between 5 and 25 ms after stimulus onset.

***M1 inactivation*:** To elucidate the causal contribution of the M1 to the muscle responses induced by the VM stimulation, muscimol was injected into the M1 hand area. As a control condition, saline was injected at the same sites and the same volume as muscimol.

Results

Part 1. Neuroanatomical experiment

To investigate the possible linkage of the transsynaptic VM–spinal pathway, two monkeys (Monkey F and Monkey L) received rabies injections into the cervical enlargement (C6–T1). Labeled neurons in the VM were widely distributed throughout the entire rostrocaudal extent of the VM including all three of the mesencephalic dopamine (DA) cell groups, the VTA, SN and RRF. The caudal part of the VM such as caudal SN and RRF contained larger number of labeled neurons than the rostral VM such as VTA and rostral SN. In addition, both contralateral and ipsilateral to the injection side have labeled neurons. This tendency was commonly observed in two monkeys.

Part 2. Electrophysiological experiment

To elucidate spatiotemporal dynamics of neuronal responses to the VM stimulation, I stimulated 31 sites in the VM in Monkey D and 5 sites in Monkey T and recorded cortical responses from frontal cortices. All stimulation sites could evoke significant responses in the OFC, LPFC, PM and M1 at 100, 300 and 500 μA. Three pulses electrical stimulation of the VM evoked short-latency negative responses in the ECoG recordings in the OFC, LPFC, PM and M1 immediately after stimulus onset (~ 5 ms). This result indicates that the VM has functional connectivity with frontal cortices including motor-related areas, and suggest that the VM could modulate cortical activities not only in prefrontal cortex but also in motor-related areas.

The VM stimulation also induced responses in multiple muscles contralateral

to the stimulation sites regardless proximal and distal muscles in 17 out of 31 stimulation sites (54.8 %) in Monkey D and 2 out of 5 stimulation sites (40 %) in Monkey T, respectively. The timing of the muscle responses followed the M1 responses. Onset latencies were distributed mainly in the range of approximately 10 – 20 ms. Since the VM had the direct anatomical projection to the M1 (Gasper *et al*., 1992; Williams and Goldman-Rakic, 1998) and the VM stimulation evoked M1 responses, the M1 might contribute to these evoked muscle responses.

To clarify whether the M1 mediates the muscle responses evoked by the VM stimulation, I performed focal inactivation of the M1 forelimb areas by microinjections of muscimol and compared evoked muscle responses before and during M1 inactivation. Muscimol was injected into the wrist and digit areas, where identified by ICMS mapping. Evoked muscle responses by the VM stimulation were disrupted during M1 inactivation. As a control condition, I injected saline into the same sites with the muscimol injection sites. Evoked muscle responses by the VM stimulation were not affected after saline injection. This result indicates that the M1 causally mediates the muscle responses evoked by the VM stimulation and suggests that the VM–spinal pathway is relayed by the M1.

Discussion

The present study demonstrated the existence of the VM–M1–spinal pathway. In reward prediction (Schultz *et al*., 1997, 1998) and processing motivation to obtain reward (Matsumoto and Hikosaka, 2009), DA neurons in the VM are functionally important. In competitive sports, up-regulation of motivation is thought to be critical for enhancing performance. Tod *et al.* (2003) has suggested that psyching-up may enhance performance requiring force production such as strength and power. In sports that crucially depend on maximum motor output, such as weightlifting, instantaneous muscle activations are required. The present result showing the facilitation of outputs to muscles via the VM–M1–spinal pathway might be a candidate for neural substrate underlying motivational control of motor outputs. Furthermore, in rehabilitation after neural damage, it is generally thought that motivation to engage in rehabilitative training is important for functional recovery. It has been demonstrated that functional connectivity among the motivation-related brain areas and the M1 are strengthened during recovery after SCI (Nishimura *et al*., 2011). Thus, the VM–M1–spinal pathway may contribute to accelerate recovery after neural damage.

In conclusion, I propose that the VM can boost motivation and motor outputs simultaneously. Further studies are needed to clarify the functional role of the VM–M1–spinal pathway during motor performance, motor leaning and rehabilitation for motor impairment.

Part II

Title: Causal role of the ventral striatum for the recovery of finger dexterity after spinal cord injury

Introduction

One of the most serious problems for individuals with motor paralysis after neuronal damage such as spinal cord injury (SCI) or stroke is deficit in dexterous finger movements such as precision grip. A great deal of effort and extensive rehabilitation are required to restore the finger dexterity. Brodal, in his literature describing his subjective experience of recovery after stroke, mentioned that "mental energy" was closely coupled with severity of his paretic muscles (Brodal, 1973). Depression is a common psychological problem after neuronal damage, and it has been shown to impede the functional recovery (Chemerinski *et al.*, 2001; Saxena *et al.*, 2007). Therefore, motivation-driven effort might be a key issue for facilitating recovery process. However, the neuronal substrate underlying such psychological effects on functional recovery remains obscure.

The ventral striatum (VSt) is a key brain region mediating a variety of behaviors requiring motivation-driven effort (Aberman and Salamone, 1999; Schmidt *et al.*, 2012). A recent non-human primate study with SCI made at the C4/5 segment has demonstrated that the VSt up-regulates the neuronal activity of the primary motor cortex (M1) and becomes to directly control finger movements during early recovery stage after SCI (Sawada *et al.*, 2015). Although they revealed that VSt had a functional role in controlling dexterous finger movements after SCI, it remains unclear whether the VSt is causally involved in functional recovery of dexterous finger movements after SCI.

Dexterous finger movements (e.g., precision grip) are severely impaired immediately after CST lesion (Lawrence and Kuypers, 1968, Nishimura *et al.*, 2007a,

2009; Higo *et al*., 2009) while coarse finger movements such as whole-finger grip recover earlier than the precision grip or remain unaffected by the injury. These observations imply that dexterous finger movements might be more demanding and require higher effort than whole-finger grip.

According to the above-mentioned findings, the VSt is suggested to be more essential for the recovery of highly demanding movement such as precision grip than less demanding whole-finger grip. To clarify the critical involvement of the VSt in the recovery of highly demanding movements, I first compared the neural substrates underlying the control of precision grip and whole-finger grip before and after SCI, respectively, by imaging the movement-related brain activity with positron emission tomography (PET). Furthermore, to investigate the causal contribution of the VSt to the motor recovery after SCI, I made bilateral lesions of the VSt by ibotenic acid injection and observed recovery time course after SCI.

Methods

***Subjects*:** Nine macaque monkeys were used for the present study. Three monkeys (Monkey H; *Macaca fuscata*, Monkey K and TF; *Macaca mulatta*; body weight 6.5-8.1 kg) were used for the PET experiment. Three monkeys (Monkey Ju, Na, and Sh; *Macaca mulatta*; body weight 3.6-4.9 kg) were used for the VSt lesion experiment (VSt lesion group). Three monkeys (Monkey M, T and R; *Macaca fuscata*; body weight 5.2-7.1 kg) were used for the VSt sham lesion experiment (Control group).

Part 1. PET experiment

***Subjects and behavioral test*:** Monkey H, K and TF were used for the PET experiment. To assess the finger dexterity, the monkeys were trained to reach, grasp and retrieve a small piece of food attached to a pin inserted through the bottom of a horizontal tube positioned in the midsagittal plane (Pin task). In addition, the monkeys were also trained to perform the same movement sequence, retrieve a small piece of food through a narrow vertical slit, which guides the monkeys to perform a precision grip (Slit task). The definition of the successful precision grip was grasping and retrieving a food morsel with the pads of the index finger and thumb (i.e. index finger-to-thumb opposition) without dropping it. The coarse grip was defined when the monkeys retrieved a food morsel without dropping it with other gripping strategies such as whole-finger grip, holding a morsel between the pad of the index finger and nail of the thumb, and raking a morsel with the index finger. Each testing session consisted of 30 trials. The rates of the precision grip and the coarse grip were calculated as the number of successful trials divided by 30.

Both behavioral test and training session were executed before and after SCI. The monkeys received lateral corticospinal tract (l-CST) lesion at the border between the C4 and C5 segments (C4/C5) on unilateral side under anesthesia after behavioral data were obtained in the intact stage.

***PET scans*:** A series of PET scans for measurements of the regional cerebral blood flow (rCBF) as an index of the neuronal activity was conducted before the injury and during the early and late recovery stages (approximately 1 month and 3 months after SCI, respectively).The monkeys were trained to perform the precision grip task and the whole-finger grip task with a constant interval (once per 5s) with the affected hand by SCI, respectively. Furthermore, they were trained to the control task, in which the food morsel stuck at the tip of the rod attached to a long tube was given to their mouth under restriction of both arms with the same pace as the reach and grasp task (see Nishimura *et al.*, 2007b). During the scan, the monkeys performed a series of reach-grasp-retrieve-eat movements every 5 s. The PET scans were performed when each monkey reached similar performance level, which was determined by the recovery level of the precision grip in the slit task.

***Data Analysis for PET*:** PET images obtained from the scan sessions were used for statistical analysis. The significant foci of inter-subject data were assessed by the analysis of covariance (ANCOVA) with global normalization using statistical analysis of the parametric mapping (SPM8) software. In order to localize the activity that reflected functional recovery, I defined the contrast as (precision grip/whole-finger grip task at the recovery stage - control task at the recovery stage) - (precision grip/whole-finger grip task

at the intact stage - control task at the intact stage). For functional connectivity analysis, the regions where rCBF values were correlated with those of the co-M1 or co-VSt during the precision grip task or the whole-hand grip task were also determined by SPM analysis.

Part 2. VSt lesion experiment

***Subjects and behavioral test*:** All the six monkeys [Monkey Ju, Na, Sh (VSt lesion group) and Monkey M, T, and R (Control group)] were intensively trained to reach, grasp and retrieve a morsel through a narrow vertical slit using both the index finger and thumb (Slit task) with left hand to assess the ability to control the dexterous finger movements as described above. Both behavioral test and training sessions were performed before and after the bilateral VSt lesion or sham lesion both before and after SCI. The data from the three monkeys of the control group were obtained from the previous study by my group (Sawada *et al.*, 2015).

***Bilateral VSt lesion*:** The monkeys in the VSt lesion group were received the bilateral VSt lesion before SCI. Multiple injections of ibotenic acid were performed to make the permanent lesion of the whole VSt (9 sites/hemisphere, 1 μl/site). The extent of the VSt lesion in each subject was defined by the area of gliosis after histological processing. As a sham lesion of the VSt, muscimol was used for the control group. To make the sham lesion of bilateral VSt in the control group, muscimol was injected into bilateral VSt of the monkeys (8 sites/hemisphere, 1 μl/site) before SCI. After behavioral data before SCI were obtained before and after VSt lesion or sham lesion, all six monkeys received the l-CST lesion on the left side.

Results

Part 1. PET experiment

Three monkeys (Monkey H, TF, and K) were tested on the PET experiments. Finger movements were impaired immediately after the SCI. In the pin task, the coarse grip such as whole-finger grip was observed immediately after SCI and disappeared in about 3 months. On the other hand, the precision grip started to recover from postoperative day 10 and fully recovered in about 3 months by rehabilitative training. Thus, grasping strategy changed from the coarse grip to the precision grip. In the slit task, the monkeys showed full recovery of the precision grip within 1 month.

To clarify the difference in the brain activation between the precision grip task and the whole-finger grip task as a representative of coarse grip, I analyzed the rCBF during the precision grip task and during the whole-finger grip task before and after SCI. The contralesional M1 (co-M1) increased its activity during both grip tasks after SCI ($P < 0.01$, uncorrected). However, the VSt increased its activity only during the precision grip task after SCI ($P < 0.01$, uncorrected).

To understand the large scaled reorganization associated with functional recovery, I performed correlation analysis in the whole brain level. During the precision grip task, significant positive correlation with the co-M1 was found in the bilateral VSt in the recovery stage, but not in the intact stage ($P < 0.01$, uncorrected). In contrast, there was no significant correlation between the co-M1 and the VSt during the whole-finger grip task both before and after SCI. Furthermore, results showed that during the precision grip task in the recovery stage, more intense functional connectivity among the cortical-

and subcortical- motor-related areas and the VSt emerged as compared to that during the whole-finger grip task in the recovery stage. This result suggested that the VSt-motor-related network underlies the performance of precision grip during the recovery process.

Part 2. VSt lesion experiment

To clarify the causal role of the VSt in functional recovery of finger movements after SCI, I made a permanent lesion of bilateral VSt before SCI in three monkeys (VSt lesion group) and compared with the cases of sham lesions of VSt in other three monkeys (control group). The lesion areas completely covered the VSt in both hemispheres. I first tested the effects of the permanent lesion of VSt on finger dexterity. None of monkeys of both the lesion and sham groups of monkeys showed impairment of the finger dexterity. All the six monkeys were then subjected to SCI. Lesion areas of the spinal cord were similar in both groups. Precision grip was impaired immediately after SCI in both groups. The control group showed recovery of the precision grip within 60 days by daily rehabilitative training, while the precision grip in the VSt lesion group was not recovered by daily training for 2 months. However, the monkeys in the VSt lesion group gradually showed recovery of the coarse grip.

Discussion

The present study demonstrated that the VSt showed functional connectivity with motor-related networks in the performance of precision grip during the recovery process after SCI. Furthermore, the permanent lesion of the VSt impeded the recovery of precision grip, indicating the causal role of the VSt in functional recovery of finger dexterity.

A recent study has demonstrated that during the early recovery stage reversible inactivation of the VSt caused a transient deficit of amelioration in finger dexterity obtained by rehabilitation. The same manipulation caused no remarkable deficit during the pre-SCI period and the late recovery stage (Sawada *et al.*, 2015). This previous study demonstrated the involvement of the VSt in dexterous finger control during early recovery stage, but the causal role of the VSt in recovery remains elusive. My result in the VSt-lesioned monkeys showed impairment of precision grip during early recovery stage (about 1 month after SCI) which was resembled with the previous study (Sawada *et al.*, 2015). However, its impairment maintained throughout observation period for 2 months when control monkeys showed recovery of precision grip. This result demonstrated that the VSt was essential for not only controlling of precision grip per se but also for recovery of precision grip.

It has been demonstrated that early rehabilitative training after SCI (Winchester *et al*., 2005; Sugiyama *et al*., 2013) or brain injury (Biernaskie *et al*., 2004) positively influences subsequent functional recovery of finger dexterity. Repetitive training on digit-use manual dexterity induces the M1 neuronal plasticity (Nudo *et al*., 1996), if applied

early but not late after injury. Transient increases in the expression of plasticity-related molecules have been reported in sensorimotor cortices (SMC) including the M1, PMv, S1 during early recovery period after SCI (Higo *et al.*, 2009). Furthermore, during early recovery stage after SCI, the VSt up-regulates activity of the SMC and is directly involved in the control of finger movements (Sawada *et al.*, 2015). Thus, starting rehabilitative training early is very important for inducing plastic change in motor-related areas and functional recovery. From these evidences, during early recovery stage the lack of facilitation of the motor-related areas by the VSt due to its lesion might prevent the reorganization in the motor-related network required for functional recovery of precision grip. Furthermore, my brain imaging results indicate that the VSt activity affect not only M1 but also other motor-related regions, and might be critical to strengthen the motor-related networks required for the recovery of precision grip after SCI. In contrast, the coarse grip recovered even in the VSt lesioned monkeys. This would be related to the less effect of the VSt on the reorganization of the motor-related network required for a whole-finger grip throughout recovery stage.

The present findings suggest that psychological and/or pharmacological approaches targeting the up-regulation of VSt can be possible treatments to promote functional recovery after neuronal damage.

General discussion

Up to today, many scientists have clarified the involvement of the mesolimbic system in motivated behaviors. However, the neural mechanism how the mesolimbic system drives or regulates motor outputs had been unclear. From results of Part I and II, I demonstrated the functional role of the mesolimbic system in motor control and the neural substrate bridging the mesolimbic system and the motor network. Together with classical interpretation of the mesolimbic system as a motivation-/reward-center, I propose that the mesolimbic system can be a critical node which can regulate both motivation and motor outputs simultaneously. My findings might be scientific evidences giving an explanation for suggestions, “psyching-up” (Tod *et al.*, 2003) and “mental energy” (Brodal, 1973) are required for motor outputs in demanding situations.