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Ventral striatum links motivational and motor networks during operant-conditioned movement in rats

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ABSTRACT

Voluntary actions require motives. It is already known that the medial prefrontal cortex (MPFC) assess the motivational values. However, it remains unclear how the motivational process gains access to the motor execution system in the brain. Here we present evidence that the ventral striatum (VS) plays a hub-like role in mediating motivational and motor processing in operant behavior. We used positron emission tomography (PET) to detect the neural activation areas associated with motivational action. Using obtained regions, partial correlation analysis was performed to examine how the motivational signals propagate to the motor system. The results revealed that VS activity propagated to both MPFC and primary motor cortex through the thalamus. Moreover, muscimol injection into the VS suppressed the motivational behavior, supporting the idea of representations of motivational signals in VS that trigger motivational behavior. These results suggest that the VS-thalamic pathway plays a pivotal role for both motivational processing through interactions with the MPFC and for motor processing through interactions with the motor BG circuits.

1. Introduction

Voluntary actions are often initiated to obtain eventual rewards or to avoid punishment. Animals can learn to execute specific voluntary actions, depending on the contingencies across cues, actions, and consequences. This is called operant conditioning, which is a fundamental form of learning goal-directed actions that allows animals to adapt to unfamiliar environments. However, it remains unclear how the motivational processes gain access to the motor execution system in the brain during operant behavior.

The limbic system plays a central role in motivational processes (Ikemoto et al., 2015; Ikemoto and Panksepp, 1996; Jennings et al., 2013; Koob, 1992; Wise, 1978; Wise and Bozarth, 1987). The nucleus accumbens (NAcc) is the core of the ventral striatum (VS), receiving direct afferents from limbic structures. The NAcc in turn projects to limbic areas and the ventral pallidum of the basal ganglia (Ikemoto and Panksepp, 1999). Based on this anatomy, the NAcc is considered a link between the limbic system and the basal ganglia (BG) (Graybiel, 1976; Mogenson et al., 1980; Ikemoto et al., 2015). The ventral pallidum projects to mediodorsal thalamic nuclei (Baleydier and Mauguiere, 1980; Jürgens, 1983), which connect with the medial prefrontal cortex (MPFC) in rats (Morici et al., 2015). In particular, the prelimbic cortex, a part of MPFC in rats, topographically connects with the VS (Berendse et al., 1992) and might encode action-outcome contingencies (Corbit and Balleine, 2003).

The limbic-BG circuit is functionally segregated from the motor-BG circuit (Alexander, 1986). This anatomical model has led to an influential serial processing theory in which motivational information is processed first in the limbic circuit and then transferred to the motor BG circuits. However, recent evidence indicates that fibers from various prefrontal cortical areas overlap substantially within the striatum (Averbeck et al., 2014; Haber et al., 2006; Haber and Knutson, 2010).

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This anatomical convergence may allow links between reward-related processing and motor-related processing in an integrative rather than serial manner (Kupferschmidt et al., 2017). Collectively, it seems reasonable to assume that the VS plays a pivotal role in operant behavior for motivational processing through interactions with the MPFC and for motor processing through interactions with the motor BG circuits.

However, direct evidence for the hypothesized network organization is scarce. The paucity of evidence partially results from technical difficulty in measuring neural activation at the whole-brain level in animals performing an operant conditioning task. Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is an established imaging technique, which can measure cerebral glucose metabolism as a surrogate marker of overall synaptic and neural activity (Phelps et al., 1981). Once ^{18}F-FDG reaches the brain, ^{18}F-FDG is taken up by astrocytes close to the sites of neural activation (Bélanger et al., 2011). Then, ^{18}F-FDG is phosphorylated by hexokinase as is glucose but cannot be metabolized further because of the lack of hydroxyl group, yielding trapped ^{18}F-FDG within the brain tissue after neural activity for an extended period. This slow kinetics of FDG makes it possible to insert a delay period between tasks and PET scanning (Endepols et al., 2010; Marx et al., 2012; Xi et al., 2013). Thus, ^{18}F-FDG-PET enables us to reveal changes in glucose metabolism during operant behavior via a delayed scanning technique.

Here we combined an operant training system and ^{18}F-FDG PET to reveal changes of glucose metabolism at the whole-brain level during operant behavior in rats. Using this method, we tested a hypothesis that VS might play a pivotal role connecting between motivational and motor systems during operant behavior. We further tested a causal relationship between neural activities in VS and motivational actions, using a pharmacological blocking method.

2. Methods

2.1. Experimental design

Sixty-four male Long-Evans rats (8-week-old at the beginning of the training; 228 ± 29 g body weight [mean ± standard deviation (s.d.)]) were used in this study (Institute for Animal Reproduction, Kasumigaura, Japan). The rats were kept under inversed light schedule (lights off at 9:00 a.m. and lights on at 9:00 p.m.) in their home cages and were handled by an experimenter for habituation (10–15 min). All animal experiments were performed under approval of the Animal Care and Use Committee of the National Institute of Neuroscience at the National Center of Neurology and Psychiatry (2014019) and were performed in accordance with guidelines for the care and use of laboratory animals. Experiments were reported according to the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines.

Among the 64 rats, 27 performed the behavior experiment only. Among the remaining 37 rats, 21 underwent ^{18}F-FDG-PET scans. Ten were assigned to the control group and 11 to the operant conditioning group. The remaining 16 rats were included in a blocking experiment that used muscimol after operant training was complete. Of these 16, 6 were in the vehicle group, 5 in the unilateral muscimol group, and 5 in the bilateral muscimol group. The 10 rats in the PET control group underwent a PET scan without operant training. Thus, 54 of the 64 rats were trained on an operant conditioning task over three consecutive days (27 in the only behavior test, 11 in the PET study and 16 in the blocking experiment). Immediately after training on Day 3, the 11 rats in the PET training group underwent the ^{18}F-FDG-PET scan. For the other 16 rats, the blocking experiment was conducted the following day (Day 4).

2.2. Animal preparation

Under 2.0%–2.5% isoflurane anesthesia, the rats in the training group underwent surgery for a dedicated head-attachment, which was made from poly-ether ether ketone (PEEK) and was attached to the skull with tiny anchor screws (PEEK, M1.2, 2.5 mm long) and dental resin (Super-Bond C & B, Sun Medical; Panavia F2.0, Kuraray Medical; UnifastII, BC Corporation, Japan). This method was similar to that in a previous study (Kimura et al., 2012), but the devices were custom-made for PET acquisition (Hori et al., 2016). During the surgery, body temperature, heart rate, breathing rate, and oxygen saturation were monitored. After recovery from the surgery, the rats were deprived of drinking water for two days before the start of behavioral training. Body weight was checked every day to monitor the status of the rats, and water was given if necessary to maintain >80% body weight (Kimura et al., 2012). The head-fixed devices were not attached on the head of the control rats because we wanted to avoid that the rats feel unnecessary painful in terms of ethical issue. Because the head-fixation device used is made of acryl resin, it is considered that there are few effects of it on the PET images (Hori et al., 2016).

2.3. Operant learning

Operant training was performed at least one week after the surgery to attach the head-fixed device. A behavioral task system (Task Forcer R1, O'hara & CO., LTD, Tokyo, Japan) was used to train rats in the operant conditioning group to perform an operant conditioning task (Kimura et al., 2012). A rat was fixed to the task system with the custom-made head attachment mounted on the head, and a spout-lever was positioned in front of its mouth. The rat held the spout-lever with its right forelimb. The rat then started to learn how to use the spout-lever via operant conditioning (Fig. 1A). Specifically, once each trial was started, a cue sound was presented to the rat (4-kHz pure tone for 0.3 s) when the rat did not pull the spout-lever for 0.3 s. If the animal pull the lever within this wait period, the trial was aborted and counted as a “Failure A” trial. In the next step, if the rat pulled the spout-lever toward its mouth within 2.0 s from the cue sound, it was allowed to drink 0.1% saccharin water (10 μl) as a reward. Such a trial was counted as a “Success” trial. Otherwise, the trial was aborted and counted as a “Failure B” trial. Subsequently, trials began within 2.8–3.2 s after the end of the previous trial. The rats were trained with this task over three consecutive days (2–3 h/day). The total amount of water intake was measured as an index of the desire for water. If we assume that the thirst was the same in all rats at the beginning, the water intake is an objective measure of how well the rats have already satisfied their need. In summary, Failure A indicates the trials in which rats pulled the lever after trial was started, so that cue sound was not presented to the rat. Failure B indicates the trials in which cue sound was presented to the rats because rats did not pull the lever for 0.3 s, but rats did not pull the lever within 2.0 s from the cue sound. Success indicates the trials in which rats pulled the lever within 2.0 s form the cue sound.

Ideally, the rats in the control group should have been fixed to the same training system used for the training rats without operant conditioning. However, in a pilot study, when we fixed rats to the training system without operant training for a while, they continued to make ballistic whole-body movement to escape from the fixation system. Thus, we avoided using the training system for the control group and instead used the restrainer (KN-326-3, Natsume Seisakujo Ltd., Tokyo, Japan) to make them to be rest for 30 min.

2.4. PET study

For ^{18}F-FDG-PET, we used a PET scanner for small animals (Clairvivo, Shimadzu Corporation, Kyoto, Japan), the specifications and physical performance of which have been reported previously (Mizuta et al., 2008; Sato et al., 2016).

A transmission scan was acquired for attenuation correction using an external ^{137}Cs source. Each rat was anaesthetized via inhalation of 2% isoflurane through a mask and was positioned prone with its brain centered in the field-of-view (FOV) of the PET scanner using the head-fixation device (Hori et al., 2016). After the transmission scan, rats in