A novel rat robot controlled by electrical stimulation of the nigrostriatal pathway

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OBJECTIVE Artificial manipulation of animal movement could offer interesting advantages and potential applications using the animal's inherited superior sensation and mobility. Although several behavior control models have been introduced, they generally epitomize virtual reward-based training models. In this model, rats are trained multiple times so they can recall the relationship between cues and rewards. It is well known that activation of one side of the nigrostriatal pathway (NSP) in the rat induces immediate turning toward the contralateral side. However, this NSP stimulation–induced directional movement has not been used for the purpose of animal-robot navigation. In this study, the authors aimed to electrically stimulate the NSP of conscious rats to build a command-prompt rat robot.

METHODS Repetitive NSP stimulation at 1-second intervals was applied via implanted electrodes to induce immediate contraversive turning movements in 7 rats in open field tests in the absence of any sensory cues or rewards. The rats were manipulated to navigate from the start arm to a target zone in either the left or right arm of a T-maze. A leftward trial was followed by a rightward trial, and each rat completed a total of 10 trials. In the control group, 7 rats were tested in the same way without NSP stimulation. The time taken to navigate the maze was compared between experimental and control groups.

RESULTS All rats in the experimental group successfully reached the target area for all 70 trials in a short period of time with a short interstimulus interval (< 0.7 seconds), but only 41% of rats in the control group reached the target area and required a longer period of time to do so. The experimental group made correct directional turning movements at the intersection zone of the T-maze, taking significantly less time than the control group. No significant difference in navigation duration for the forward movements on the start and goal arms was observed between the two groups. However, the experimental group showed quick and accurate movement at the intersection zone, which made the difference in the success rate and elapsed time of tasks.

CONCLUSIONS The results of this study clearly indicate that a rat-robot model based on NSP stimulation can be a practical alternative to previously reported models controlled by virtual sensory cues and rewards.

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KEYWORDS animal robot; nigrostriatal pathway; electrical stimulation; turning behavior
Artificial stimulation of specific brain circuits can induce stimulation-specific behavioral responses.\textsuperscript{14,15} Talwar et al. introduced a remotely guided rat robot by electrically stimulating the somatosensory cortex and reward center.\textsuperscript{3} These authors reported that after proper training, rats were able to recognize the relationship between a somatosensory cue and a reward. This rat robot was manipulated to perform complicated tasks in various mazes or open fields. Animal robots controlled via stimulation of various brain areas, such as the medial forebrain bundle,\textsuperscript{1,3}–\textsuperscript{5} ventral posterolateral nucleus thalamus,\textsuperscript{7} ventral posteromedial thalamic nucleus (VPM),\textsuperscript{7} amygdala nucleus,\textsuperscript{7} pedunculopontine tegmental nucleus,\textsuperscript{8,9} and dorsal periaqueductal gray nucleus,\textsuperscript{15} have also been reported.

However, these animal robots had inherent limitations in terms of the accuracy and time requirement for successful navigation control, since the rats had to be trained and retrained for a period of time in order to generate and maintain successful animal robots. Furthermore, the directions and angles of turning movements basically depended on the animals’ volitions.\textsuperscript{7} Thus, eliminating the time requirement for the animal’s learning and inaccurate responses is essential for the production of a successful rat-robot system. From this perspective, “prompt” movement control via stimulation of one of the motor circuits could be a more effective method to control animals. Although motor system stimulation studies are numerous, they have primarily aimed to elucidate the mechanisms of movement generation in normal and abnormal states.\textsuperscript{16–20}

The nigrostriatal pathway (NSP) is a dopaminergic pathway in the brain and is involved in the production of movement as part of the basal ganglia motor circuit.\textsuperscript{22–24} It is known that continuous electrical stimulation of the NSP in rats and cats can evoke instantaneous contraversive turning or circling movement.\textsuperscript{25–28} The induction of prompt contraversive turning movements with specific angles via appropriate bilateral NSP stimulation could be used to instantly command left or right directional movements without the need for animal training. However, this NSP stimulation–induced turning behavior has never been used to build an animal-robot system. Therefore, in this study, we aimed to electrically stimulate the NSP of rats to build a command-prompt rat robot.

Methods

Experimental Animals

Male adult Sprague Dawley rats (Samtako Bio Korea) weighing 350–450 g were individually housed in polycarbonate cages with wood chip bedding and ad libitum access to food and water. We maintained a 12-hour light/dark cycle (8:00 AM to 8:00 PM) at a constant temperature (22°C ± 2°C) and humidity (55% ± 5%). All procedures were approved by the Institutional Animal Care and Use Committee of Hallym University and were conducted to minimize the number of animals used.

Electrode Implantation

All experimental rats (n = 7) were acclimatized at least 7 days prior to surgery. For electrode implantation, a rat was placed on a stereotactic apparatus after a tail-pinchi-
conducted for each rat. In the control group, 7 rats were tested in the same manner but without NSP stimulation. Behaviors on the T-maze were also recorded using a web-cam (Fig. 1 and Video 1).

**VIDEO 1.** Behavior control in the T-maze. Copyright Hyung-Cheul Shin. Published with permission. Click here to view.

**Data Analysis**

All data are expressed as the mean ± standard error of the mean, and an unpaired t-test and Mann-Whitney U-test were used to test for differences in the turning angle, navigation time, number of NSP stimulations, components of NSP stimuli, and learning effect. Further analyses were conducted using a one-way ANOVA with Tukey’s multiple comparison or Kruskal-Wallis test with Dunn’s multiple comparison. A p value < 0.05 was considered significant, and all statistical analyses were performed using GraphPad Prism 5.03 (GraphPad Software).

**Results**

In the experimental group, the overall mean stimulation strength for both sides of the NSP was $143.93 ± 12.29 \mu A$, and there was no significant difference (p > 0.05) between left ($148.57 ± 17.52 \mu A$) and right ($139.29 ± 18.47 \mu A$) stimulations. All 7 experimental rats exhibited immediate contraversive turning movements in response to NSP stimulation (Table 1).

**Turning Angles Following Various Stimulation Intensity**

The turning angles produced by high-intensity stimulation of $160 \mu A$ (left $64.69° ± 7.12°$, p < 0.05; right $70.13° ± 5.25°$, p < 0.05) and $180 \mu A$ (left $59.50° ± 7.21°$, p < 0.05; right $78.50° ± 7.50°$, p < 0.05) were significantly larger than those generated by low-intensity stimulation ($60 \mu A$: left $39.25° ± 4.70°$, right $43.50° ± 4.92°$). The rightward, but not the leftward, turning angle elicited by $140 \mu A$ was significantly larger than that elicited by $60 \mu A$ (Fig. 2B).
Overall, the mean values of the turning angles exhibited a strong relationship with the stimulation intensities (left turning $R^2 = 0.8776$, right turning $R^2 = 0.9356$). Based on these results, the appropriate stimulation intensities to control rat movement were selected.

### Repetitive NSP Stimulation to Degrees of Turning Angles

We tested the stability of NSP stimulation to elicit turning movements after short interstimulation intervals. The results indicated that the repetitive stimuli elicited prompt turning movements and that the degree of turning movement induced by NSP stimulation at a given interval (either 1 or 5 seconds) did not change significantly across trials (Fig. 3), although the 1-second interval NSP stimulation induced significantly ($p < 0.05$) larger angles ($41.5^\circ \pm 1.26^\circ$) of turning movements than the 5-second interval stimulation ($34.40^\circ \pm 0.94^\circ$). It can be assumed that the intermittent voluntary behaviors (such as grooming, exploratory movements, etc.) that occurred during the repeated interposed periods offset the turning movements in the 5-second interval condition. As a result, either a 1- or a 5-second stimulation interval is enough to induce proper turning behavior in rats.

### Movement Control of Rats in a T-Maze Based on NSP Stimulation

The experimental group exhibited 100% successful robot-like movements by reaching the target areas (Table 1). The completion time of the first trial was not significantly distinguishable from those of subsequent trials, which revealed that there was no learning effect of NSP stimulation. The elapsed times of the first left and last right directions in the T-maze task were $14.29 \pm 2.2$ and $11.57 \pm 1.7$ seconds, respectively, which were not statistically sig-

### TABLE 1. Success rate of and time required to complete the T-maze task

| Rat No. | Direction | Success Rate (%) | Elapsed Time (sec) |
|---------|-----------|------------------|--------------------|
| 1       | Lt        | 100              | 12.20 ± 2.22       |
|         | Rt        | 100              | 12.40 ± 1.50       |
| 2       | Lt        | 100              | 18.00 ± 1.10       |
|         | Rt        | 100              | 11.80 ± 1.20       |
| 3       | Lt        | 100              | 16.00 ± 1.45       |
|         | Rt        | 100              | 9.60 ± 0.81        |
| 4       | Lt        | 100              | 18.20 ± 1.77       |
|         | Rt        | 100              | 16.00 ± 1.05       |
| 5       | Lt        | 100              | 9.00 ± 1.76        |
|         | Rt        | 100              | 8.60 ± 0.60        |
| 6       | Lt        | 100              | 9.40 ± 1.50        |
|         | Rt        | 100              | 5.60 ± 0.40        |
| 7       | Lt        | 100              | 17.20 ± 1.36       |
|         | Rt        | 100              | 17.00 ± 2.83       |

FIG. 2. The effect of varying the stimulation amplitude. A: The turning angle increased as the amplitude of the NSP stimulation increased. B: Comparison of left-turning angles elicited by each stimulus amplitude. C: Comparison of right-turning angles elicited by each stimulus amplitude. *$p < 0.05$, **$p < 0.01$. 

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nificantly different (p > 0.05; Fig. 4). Overall, 12.93 ± 0.60 seconds was required for completion of the T-maze, and the time at the intersection zone (5.61 ± 0.41 seconds) was significantly longer than those at both the start arm (3.83 ± 0.25 seconds, p < 0.05) and the goal arm (3.49 ± 0.22 seconds, p < 0.05).

For all 70 trials of the target-arm tasks, the experimental group required a significantly shorter period of time than the control group, which achieved a success rate of only 41% (Fig. 5). The amount of time spent on forward movements on the start and goal arms was not significantly different between left and right directional navigation tasks. However, a difference was observed in the intersection zone (Fig. 5B). The number of command stimuli applied from the start arm to the target arm was 18.61 ± 0.89. Interestingly, command stimuli applied at the intersection zone (9.17 ± 0.59) were significantly greater than those applied at both the start and goal arms (Fig. 5C). The left-arm task required a significantly greater number of stimuli at the intersection zone than did the right-arm task, whereas the number of stimuli required for forward behaviors at the start and goal arms was not significantly different between the left- and right-arm target tasks (Fig. 5D).

Optimal Control of NSP Model

At the intersection zone during the left-arm target task, right NSP stimulations were applied more frequently than the two other commands (left NSP 31%, right NSP 61%, both NSPs 8%; Fig. 6B); during the right-arm target task, left NSP stimulations were more commonly delivered than the two other commands (left NSP 66%, right NSP 25%, both NSPs 9%; Fig. 6E). To induce forward movements in the intersection zone for the 70 trials, rats were manipulated by either simultaneous stimulation of both NSPs or alternating left and right NSP stimulations. Three commands, i.e., stimulations to the left NSP, the right NSP, or both NSPs, were used differently to optimize movement of the rat robot on the three sections of the T-maze (Fig. 6).

Discussion

Controlling Animal Movement Using NSP Stimulation

The basal ganglia have been related in motor diseases, yet how they contribute to the production of movement remains a point of investigation. It is known that the basal ganglia are implicated in the production of contraversive movements. Previous studies reported that the concurrent balanced activity in both striatonigral and striatopallidal projection pathways was essential for the generation of contraversive movements and that imbalanced activity between the two pathways could result in ipsiversive motor output. Arbuthnott and Crow first reported contraversive turning behavior by electrical stimulation of the NSP of rats. Their stimulation sites covered medial and anterior parts of the substantia nigra. After making electrolytic lesions through electrode tips, they observed decreased dopamine content of the caudate nucleus on the same side. The turning movements were presumed to be circular ones. These contraversive turnings were observed 3 or 4 times during stimulations, and they were rarely ob-
served until 10 seconds of repetitive stimulation. In later experiments, they reported that continuous stimulation through electrodes implanted in the posterolateral hypothalamus close to the ascending axons of the nigrostriatal dopamine neurons caused rapid circling behavior toward the unstimulated side. In cat experiments, electrical stimulation to the substantia nigra also exhibited head turning movement and subsequent contraversive circling movements. Lammers et al. also reported the generation of circling behavior through stimulation of the nigrostriatal dopaminergic fibers in the hypothalamus of rats. Thus, these continuous high-frequency NSP stimulations, which produce immediate contraversive rotational movements, cannot be used as commands for the fine movement control of the animal robot.

In the present study, 7 rats successfully completed experiments for both the open chamber and the T-maze. Each stimulus caused a certain degree of angular directional turning movement, not a complete circular rotation, which ceased within 1 second after stimulation. This type of NSP stimulation–induced immediate directional turning movement at a specific angle was a necessary condition for the fine control of rat-robot navigation in our study.

Many animal-robot studies have explored the effects of electrical stimulation of various brain areas to control animals. In most of these studies, animals need to learn the relationship between virtual cue stimulation and emotion-ally assistive stimulation. Thus, the animals could not be promptly controlled by command stimulations initially. A few recent studies have reported that one could induce command-prompt animal movements by applying electrical stimulation to the VPM. However, their study mainly focused on the quantitative controllability of the turning angles dictated by varying stimulation parameters. They asserted that the VPM was a more “solid” location to stimulate in order to induce immediate evasive turning movements to the virtual sensory inputs. The notion of the solid somatosensory input at the VPM as a cue for the generation of an evasive motor movement appears to be an oversimplification of the overall tactile and kinesthetic functions of the somatosensory system. The vibrissae are large tactile sensor arrays on the rat face that are used to actively explore various objects and spatial environments. In addition, the VPM is reciprocally connected to the somatosensory and motor cortices to actively carry out sensory motor integrative functions in ever-changing surroundings to generate appropriate motor behaviors for survival. Furthermore, a rat robot manipulated by VPM stimulation had a limitation in that repetitive stimulation for at least 30 seconds was needed to induce a full range of turning behavior. To compare the stability of our NSP model, we tested whether there were any changes in the effectiveness of the NSP stimulation for eliciting turning movements by applying repetitive stimulation at two dif-
different interstimulus intervals, 1 or 5 seconds (Fig. 3). A short interstimulus interval indicates that a controller can deliver a greater number of directional commands over the same period, which ensures more accurate behavioral control of the animal robot. In our T-maze experiments, the overall mean interstimulus interval was 0.71 ± 0.002 seconds. Our results showed that each stimulation caused an immediate rightward turning movement with a specific angle and that the successive stimuli delivered at short interstimulus intervals did not cause any weakness or failure of the immediate turning movement.

**Optimal Parameters to Control Rat Movement**

In our study, we found that the angles of NSP stimulation–induced turning movements were correlated to the intensities of the stimulation (Fig. 2). Initially, we began with a stimulation intensity set to 60 μA, which was increased by 5-μA increments. If a rat turned at least 15° for an NSP stimulation or moved forward at least one step for a forward command, the response was accepted as an effective one. Among the 7 experimental rats, the overall mean stimulation strength for both sides of the NSP was 143.93 ± 12.29 μA. Forward movements were induced by either simultaneous or alternating stimulation of the left and right NSP. The results of the T-maze experiment indicated that at both the start arm (3.83 ± 0.25 seconds) and the goal arm (3.49 ± 0.22 seconds), the amount of time that the rat spent on forward movements was fairly short and the number of stimuli was relatively low (start arm 4.93 ± 0.33, goal arm 4.53 ± 0.29; Fig. 5). These findings were not significantly different between the left-arm task and the right-arm task. Navigation at this intersection zone required 5.61 ± 0.41 seconds, and the NSP stimulations had to be delivered in high numbers (9.17 ± 0.59). In the

**FIG. 6.** Three command stimulations were variously used to optimize movement: start arm for left-arm target tasks (A), intersection zone for left-arm target tasks (B), goal arm for left-arm target tasks (C), start arm for right-arm target tasks (D), intersection zone for right-arm target tasks (E), and goal arm for right-arm target tasks (F). *p < 0.05, **p < 0.001.
intersection zone, the right NSP command was used most frequently for the left-arm target tasks and the left NSP command was most frequently used for the right-arm target tasks (Fig. 6B and E). This was a predictable response, since the NSP command from one hemisphere was supposed to induce the contralateral turning movement.

Analyses of results from the T-maze raised the possibility that the rat movements were based on tactile sensations from contacting the walls of the maze in addition to the commanding NSP stimulation. To assess this possibility, we performed a navigation in a rectangular open field (Video 2).

VIDEO 2. Behavior control in the open field maze. Copyright Hyung-Cheul Shin. Published with permission. Click here to view.

The results strongly suggested that our rat robot could be well controlled by command to the NSP in an open field without use of the animal’s tactile sensations from whiskers and body.

Possible Applications and Future Directions of the NSP Model

Overall, our results indicated that a novel rat-robot model could be constructed by electrically stimulating the NSP. We believe that this training-free rat robot may overcome the major limitations of previous animal robots in terms of accuracy, ease, training, and maintenance. Future studies to refine or improve our rat-robot system may involve 1) wireless control, 2) automatic or semi-automatic selection and delivery of command types and intensity of stimulation in real time according to changing environments monitored by various mounted sensors, 3) employment of multichannel electrodes implanted in different subregions of the NSP for the selective induction of specific behavior(s), and 4) applications of this system to other animals. The rat-robot system controlled by NSP stimulation could be utilized in various industries to detect and remove dangerous items that cannot be easily accessed by humans.

Conclusions

We introduced a novel rat-robot control method using intracranial microstimulation of the NSP. In this new model, the rat robot was able to conduct tasks immediately after recovery from the implantation surgery. Furthermore, our rat robots did not need any training or maintaining procedures such as those used in previously reported behavioral control models. Therefore, the NSP stimulation method could provide more effective and accurate control of rat navigation.

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**Disclosures**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

**Author Contributions**

Conception and design: HC Shin, Koh, HY Park, Chang.

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Reviewed submitted version of manuscript: HC Shin, Koh, Chang.

Approved the final version of the manuscript on behalf of all authors: HC Shin.

Administrative/technical/material support: Koh.

Study supervision: HC Shin.

**Supplemental Information**

**Videos**

*Video 1.* [https://vimeo.com/418899211](https://vimeo.com/418899211).

*Video 2.* [https://vimeo.com/418899263](https://vimeo.com/418899263).

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