Rattractor - A Realtime Place-triggred Rewarding Stimulator for a Rat

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Abstract.

Objectives To influence animal behaviors by applying electrical stimuli in the central nervous system, we developed "Rattractor" (rat attractor), a system for electrical stimuli application in the rat brain, when the subject stays in a specified region or a virtual cage. Our results showed that our closed-loop system facilitated a self-organized control of rat locomotion to stay in the virtual cage.

Approach Nine rats had two wire electrodes implanted in the deep brain. The electrodes targeted the medial forebrain bundle (MFB), a part of the reward system in the deep brain. Following a recovery period, rats were placed on a plain field where they could move freely but wired to a stimulation circuit. An image sensor installed over the field detected the subject position which accordingly triggered the stimulator, so that rat stayed within the virtual cage. We conducted a behavioral experiment to evaluate a sojourn ratio that the rat resided in the region. Thereafter, histological analysis of the rat's brains was performed to confirm the position of the stimulated sites in the brain.

Results Seven rats survived the surgery and the recovering period without technical failures such as connector break. Then, we observed that three of them tended to stay in the virtual cage during stimulation, an effect maintained for two weeks. Histological analysis showed that the electrode tips were correctly placed in the MFB region of these rats. The other four subjects showed no apparent preference to the virtual cage. In these rats, we did not find the electrode tips in the MFB or could not determine its position.

Significance Almost half of the rats showed a tendency to stay inside the virtual cage when position-related reward stimuli were triggered in the MFB region in real time, as shown by optical observation thorough the image sensor. The experimenter only needed setting the region of the virtual cage, not requiring sequential commands

for the rat. Notably, our system neither required previous training nor sequential intervention to affect the behavioral preferences of the subjects. This process is similar to the situation wherein sheep chased by a shepherd dog in the desired direcion indicated by the dog.

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1. Introduction

Electrical stimulation in the living animals can induce virtual sensations, muscular contractions, and result in behavioral alternations. For example, Lopez et al.[1] developed various virtual reality games in which players experienced somatosensory impacts on their limbs by electrically triggered muscle contraction in virtual environments such as a boxing ring or a soccer field. Another example of this technology is "Cruise Control"[2] which providing automated muscle stimuli, allowed a person to avoid obstacles on a predefined pathway with their eyes closed. Moreover, It has been applied for posture training when pitching a bowling ball by inducing muscular contraction in the limb[3].

Electrical stimulation in the muscles also called functional electrical stimulation (FES) has been long used for clinical applications enabled patients with the paralysis or the spinal chord injuries to manipulate their disabled limbs for grabbing objects, standing up, and even stepping forward[4]. Recently, a method for bypassing injured nerve fibers and transmit efferent voluntary motor commands into the patient's own muscles[5] has been proposed.

In animal models, more direct interventions into their behaviors has been demonstrated. *Roboroach*[7, 8, 9], for example, applying stimuli in the hemi-lateral antennae has been used to manipulate cockroach locomotion into an opposite direction.

Its behavior was manipulated by an experimenter who dispatched sequential instructions according to the circumstances, similar to a radio-controlled vehicle.

On the other hand, *Ravigation* or a rat navigation system[6] affected rodent behaviors by a rewarding stimulus in the medial frontal bundle (MFB) region. Here, the subject received stimuli in the MFB, a part of the reward system in the brain. The subject was rewarded after following an instruction to make a directional turn indicated by an electrical stimulus in the sensory cortex (SCx). However, this system also required sequential instructions, and ex ante training periods for the subject to associate the stimuli in the SCx and those in the MFB. Training is not only a burden both for experimenters and subjects, but also increases the possibility of unexpected interruptions such as device defects and animal fatigues.

To avoid these problems, we developed an automated reward stimulator whereby the rat position itself triggers the stimuli. This affects animal behaviors without training and could be useful for industrial applications based on the animal behavior, like mine

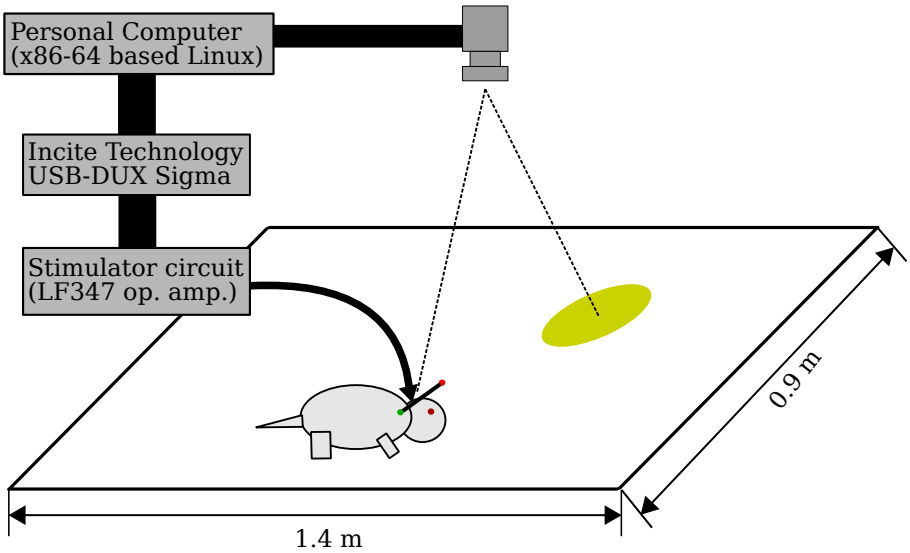

Figure 1. Appearance of the Rattractor system. The rat was stimulated in the MFB as it entered the virtual cage indicated with a circle.

exploration in minefield barrier fence, for example. We developed a system called "Rattractor" as a proof-of-concept, in which the reward system in the rat brain was stimulated when the subject stayed in a specific circular region simulating a virtual cage. While our preceding work roughly demonstrated this concept [10, 11, 12], this paper goes one step further focusing on a quantitative evaluation of the system.

2. Materials & Methods

2.1. Methodology

This section describes the general approach of the Rattractor system including animals preparation.

2.1.1. System Design

Devices The Rattractor system is essentially an electrical feedback stimulator. It stimulates the rat brain with electrical stimuli triggered by the optically observed rat position. To achieve this functionality, we placed a high-speed, low-latency camera (Basler acA800-510uc) over a plain field (1.4 m $\times$ 0.9 m) where a rat can freely explores, wanders, and rests (Fig. 1). The top surface of the field was covered with a black elastic sheet, and three edges were limited by walls also covered with elastic sheets, while the other edge faced a cliff from which rats rarely jumped out.

The camera which had XGA (1024 $\times$ 768) resolution covered the whole field including its edges, and was connected to a desktop computer (Intel Core i5 CPU; 8 GB memory; operated with Linux) by a USB 3.0 cable. After detecting the rat

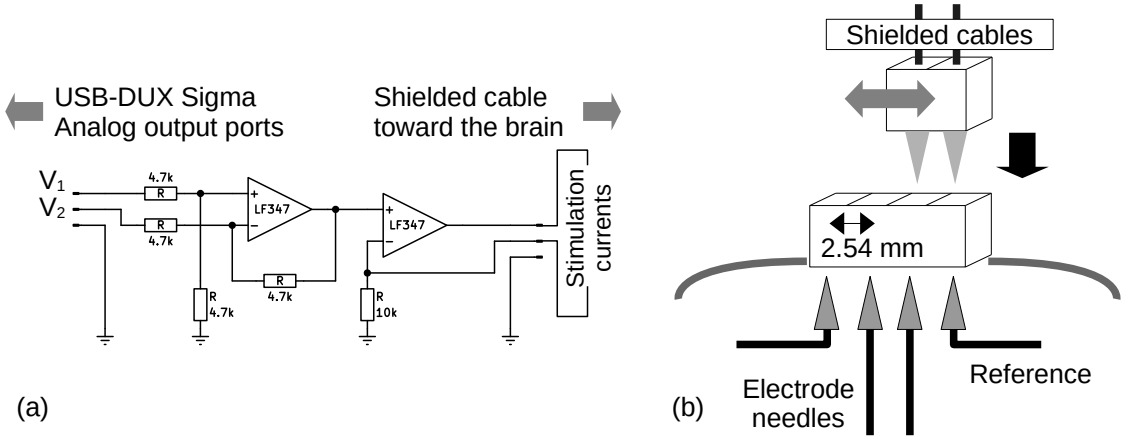

Figure 2. (a) Stimulator circuit to generate bipolar current stimuli. (b) Electrode unit with two electrode needles and two references.

position, the computer produced stimulation triggers via an analog output interface (Incite Technology, USB-DUX Sigma).

We attached a current source circuit (Fig. 2a) to the analog output connector, which consisted of a voltage differentiation circuit and a constant current supplier circuit using an LF347 operational amplifiers package to inject charges into a living animal. The circuit accepted two monopolar voltage inputs V_1, and V_2 between 0–2.5 V from the analog output board to generate currents $I = (V_1 - V_2)/10^4$ between -2.5 and +2.5 mA. It also included a shunt resistor to monitor a voltage proportional to the current.

The currents were then applied to the brain through a stimulation interface unit placed on the skull. The unit consisted of two needles and a conventional 0.1 inches (2.54 mm)-pitched connector with four lines (Fig. 2b). Each needle was clipped from a commercially available neural macro electrode (MicroProbes LF-301G) made of Tungsten. We removed polyamide coating around its end with a paper grinder, and swaged a metal contact pin on the end. Then, the needles were inserted into the second and third terminals, at an interval of 0.1 inches (2.54 mm) between the two needles. The first and fourth terminals were used to hold the reference lines. Finally, the whole structure was covered and fixed with thermoplastic adhesives.

The unit was wired to the stimulator circuit via shielded cables during behavioral experiments. Two green and red light emitting diodes (LEDs) connected by a battery (CR2032) were placed at the end of the cables, which eased tracking the position and the direction of the rat's head.

Controller A controller was implemented on the computer to dispatch stimulation triggers according to the rat's position was. Image frames were grabbed every 30 – 50 ms. Then, the green and red color filters were applied to the image, binarized, and its spatial moment calculated to obtain $\boldsymbol{r}_{\mathrm{Green}}$ and $\boldsymbol{r}_{\mathrm{Red}}$, the coordinates of the red and green LEDs, respectively.

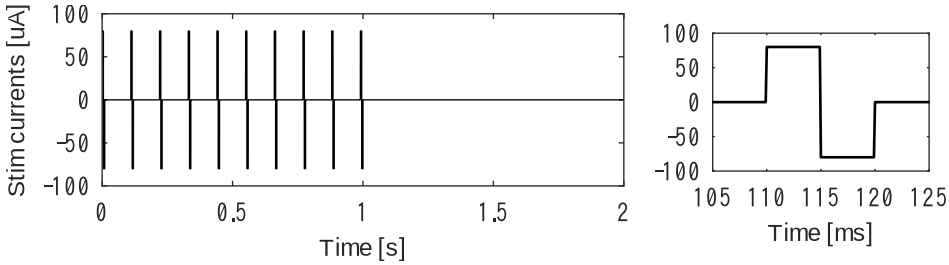

Figure 3. A set of 10 square pulses were generated with our circuit to apply bipolar current stimuli (left); temporal closeup around the first pulse (right).

The rat position was calculated as follows: $\boldsymbol{r}_{\mathrm{Rat}} = (\boldsymbol{r}_{\mathrm{Green}} + \boldsymbol{r}_{\mathrm{Red}})/2$, which triggered the stimuli as long as the rat stayed within the virtual cage. That is, stimulation was triggered as $|\boldsymbol{r}_{\mathrm{Rat}} - \boldsymbol{r}_{\mathrm{Cage}}| < 0.1$ m, where $\boldsymbol{r}_{\mathrm{Cage}}$ was the position of the virtual cage. The trigger frequency was limited to 5 Hz at the highest to avoid excess brain stimulation.

Each trigger generated a waveform consisting 10 repeating bipolar squares at 80 μV amplitude and 5 ms duration (Fig. 3).

2.1.2. Animals preparation Nine male Wistar rats were prepared for the experiment. They were bred by an animal provider (Tokyo Laboratory Animals Science Co., Ltd.) in a clean/SPF-graded environment until delivered to our facility. At this point, they weighted 250 g and were about 10-weeks-old. They were able to drink water freely in the cages but were fed with a restricted amount of solid food to maintain their weights until surgery, typically conducted 1–4 weeks after arrival.

The surgery to implant the electrode unit was conducted as follows. First, rats were anesthetized with isoflurane gas at 2.0 – 2.5 %, and kept immobilized at 1.0 – 1.5 % until the end of the surgery. After applying Xylocaine jelly local anesthesia on the parietal skin, the rat was fixed to a stereotaxic brain surgery device. Then, we applied isodine on the skin followed by Maskin hydrochloride for sterilization while waiting for the local anesthesia to be effective. Subsequently, we incised the skin and subcutaneous tissues with a surgical blade and drilled holes on the skull to implant both electrodes and anchors.

Two holes were drilled at 1.3 mm and 3.7 mm posterior, and 2.0 mm right lateral from the *bregma* skull joint using a hand drill with a 1.2 mm bit. These holes directed the needles into the MFB which is approximately found along the body axis in deep areas of the brain. In addition, depending on the condition of the skull surface, 6–8 holes were drilled to hold anchor screws.

Finally, the electrodes unit was placed over the two holes in the skull with a stereotaxic manipulator, and directed ventrally in the brain until reaching depth of 8 mm from the brain surface.

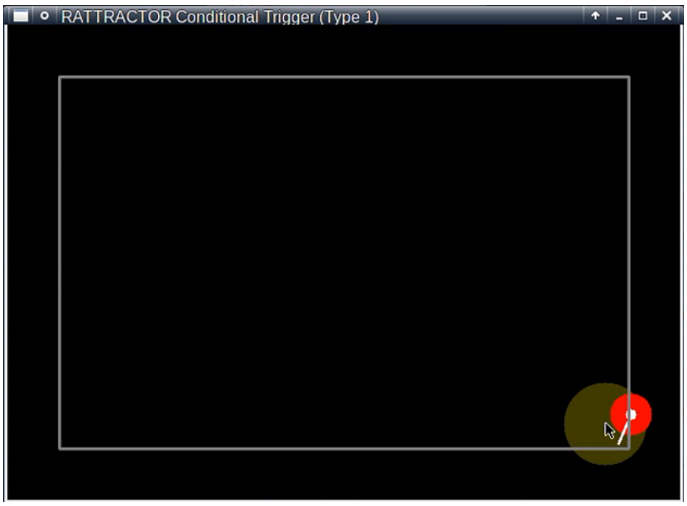

Figure 4. A graphical user interface (GUI) was developed to show the rat's position (white circle) and heading direction (bar) as well as the stimulated region (i.e., virtual cage), drawn as a dark yellow circle and manually positioned by the experimenter using the mouse cursor. The red color around the rat position indicates that the subject is electrically stimulated at that time.

2.2. Experimental procedures

2.2.1. Behavioral Experiments The behavioral experiments were conducted at one week (Post-operation week 1; POW 1) and two weeks (POW 2) after surgery. All rats, participated in POW 1 experiments, except for subject D, whose connector accidentally detached during the recovery period. Based on their preference to stay in the virtual cage in the POW 1 session, three subjects (Subject C, F, and I) further proceeded to POW 2 experiments.

The experiment began by wiring the rat with to allow providing electrical stimuli to the brain. When the cables were connected to the two forward lines of the unit, the anterior MFB site was stimulated against a reference placed on the skull. Similarly, the posterior MFB site was stimulated against a reference placed on the skull by connecting the cables to the two rear lines of the unit.

Then the rat was left to move freely on the field for 10 min for habituation. Most subjects showed exploratory behavior until relaxing on the corner of the experimental field. Subsequently, we set a virtual cage region with our GUI (Fig. 4), typically in front of the rat's nose so that the rat could enter the cage only with a single voluntary step.

We prepared two conditions; the first *fixed* condition placed the virtual cage in the initial position for 10 min, and the second *moving* condition moved the virtual cage straight from the initial position toward the far corner of the field. The *moving* condition was applied only if the stimuli appeared in the *fixed* condition appeared effective.

2.2.2. Histology After behavioral experiments, the rat was deeply anesthetized with pentobarbital sodium and electric lesions were burnt with a 0.2 mA current to mark the

electrode position. The brain was removed and stored in buffered formalin (10%) for 7 days; coronal sections (100 μm) were cut using a micro-slicer (DTK-1000, DOSAKA EM, Japan) and stained with cresylviolet.

2.3. Analysis

We analyzed both behavioral and histological results in two steps.

First, the rats were first screened into two groups according to the stereotaxic coordinates of the electrode tips in the brain histologically observed after sacrificing the rats. The subjects in whom the electrode tips were found in MFB regions were sorted into the *positive* group and their behavioral results in POW 1 & POW2 analyzed. Other subjects, in which we failed to find the electrode tips in the MFB were placed in the *negative* group and their behaviors only analyzed on POW1 as a reference session without reward stimuli.

Then, we defined a distance metric $d = |\boldsymbol{r}_{\mathrm{Rat}} - \boldsymbol{r}_{\mathrm{Cage}}|$, where $\boldsymbol{r}_{\mathrm{Rat}}$ and $\boldsymbol{r}_{\mathrm{Cage}}$ was the horizontal coordinate of the head position and of the center of the virtual cage, respectively. These metrics were calculated on every image frame at a time stamp k ($k = 1, \ldots, K$) captured by the camera for each subject (A, B, ..., and H). In addition, another condition with stimulated sites was evaluated. The stimulated sites were either the anteior, posterior, or the null site, where currents were shunted.

The first 210 s (3 min and 30 s) part of each session ($t_k = [0, 210]$) was extracted to compare $d(t_k)$. The ratio of frames count during which the rat stayed within the virtual cage was calculated as,

$$\gamma = \frac{1}{K} \sum_{k=1}^{K} \delta \left(d(t_k) < 0.1 \right),$$

where $\delta(\cdot) = 1$ for a true condition and otherwise $\delta(\cdot) = 0$, was calculated to compare the efficacy of attracting the rat between conditions, i.e., subjects and stimulated sites.

In addition, in POW 2, we moves the virtual cage tugging the subject C to an arbitrary location. In said session, the virtual cage moved from one corner to the opposing corner of the field, and back to the center of the field.

2.4. Remarks

Animals preparation and experimental procedures described in this section were approved by Animal Experiments Commitee of Graduate School of Medicine, the University of Tokyo, following their guidelines.

3. Results

3.1. Screening

We inspected rat's appearance and behavior after the surgical procedure, to determine whether the subject was suitable for the subsequent experiments.

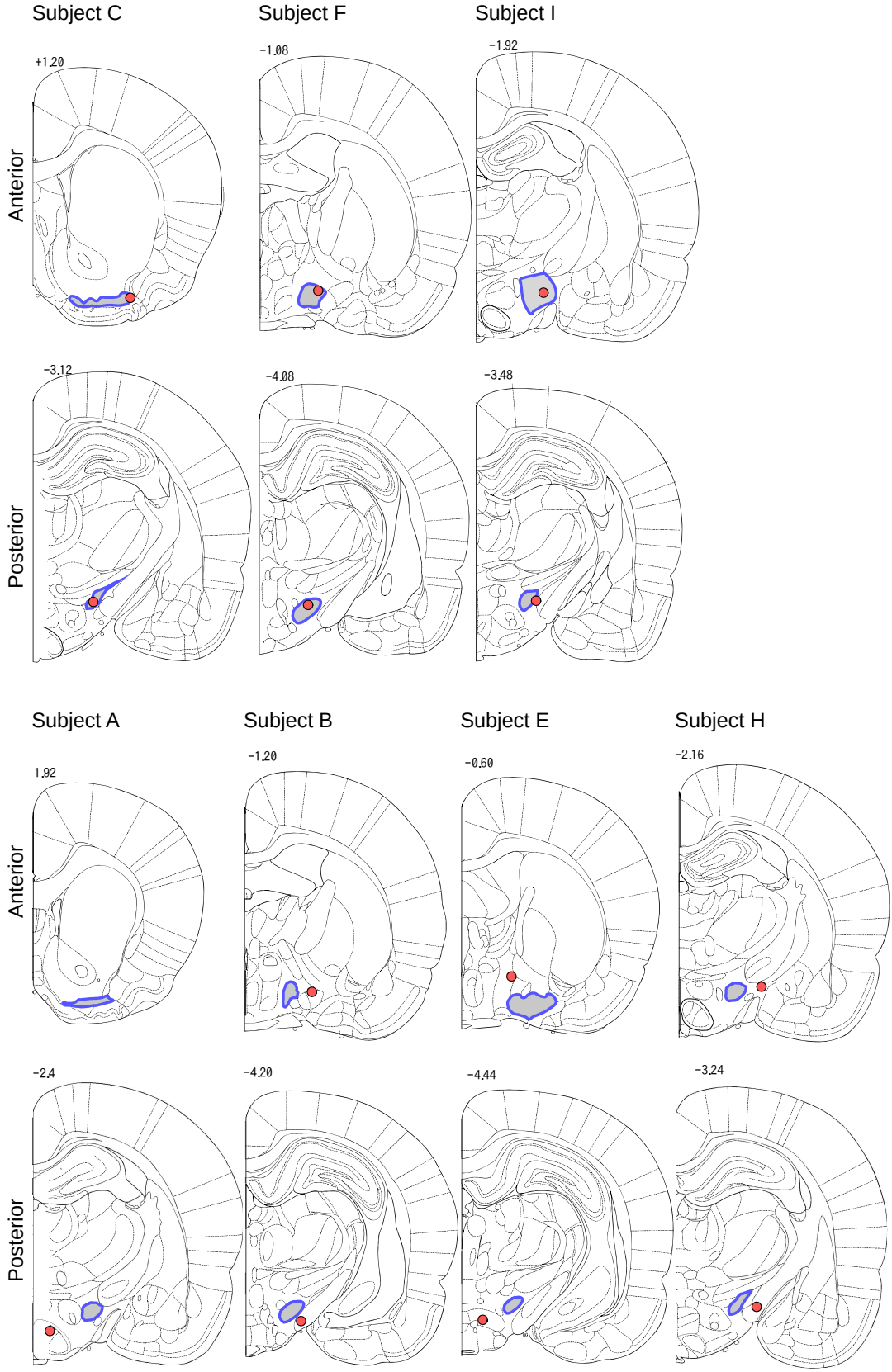

Figure 5. In each image, a red circle indicates the stimulated site and the gray area surrounded by a blue outline highlights the MFB region superimposed on a coronal section of a stereotaxic atlas image.

The behavioral screening focused on abnormal behaviors such as violent or fear responses during and after the electrical stimulation. We found no remarkable abnormal behaviors instantly caused by the stimuli. In addition, there were no persisting behavioral changes after stimulation.Therefore, all subjects took part in the following behavioral test sessions.

However, we removed several subjects from behavioral analysis due to misplaced electrodes in the brain, as determined by histological screening after completion of behavioral experiments.

Figure 5 indicates the positions of the electrode tips in the rat brain superimposed on a coronal section of a stereotaxic atlas map, where the MFB region is indicated by a gray area surrounded by a blue outline.

We found clear evidence that the electrode tips for both anterior and posterior stimulation sites were located within the MFB in the subjects C, F, and I, while other subjects A, B, E, and H the lesions were not located within the expected region. Both the anterior and posterior stimulation sites were found far out of the MFB in subjects B, E, and H. In subject A, the posterior site was located outside of the MFB; its anterior site could not be determined in the observed sections.

Consequently, we categorized subjects C, F, and I in the *positive* group, and subjects A, B, E, and H in the *negative* group, which were only tested in POW 1.

3.2. Typical behaviors

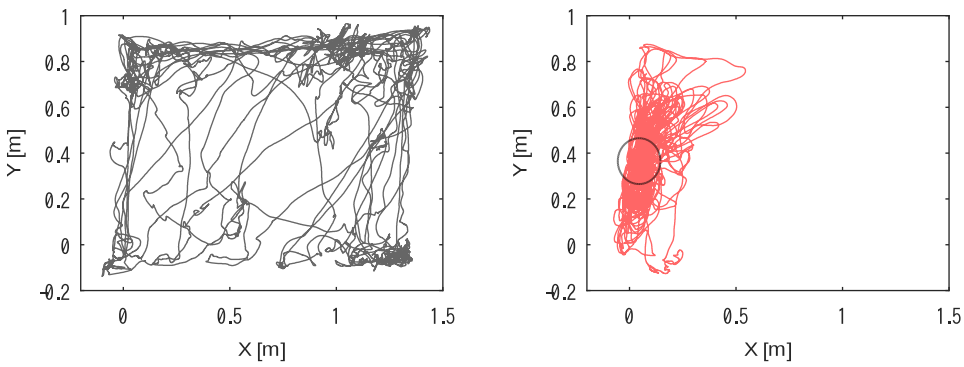

Figure 6. Typical rat trajectory without electrical stimulation in the brain (null condition; left), and a trajectory under stimulation in the MFB as the rat stayed in the virtual cage indicated by a circle (right).

Figure 6 shows typical rat locomotion trajectories. The rat moved all over the field, mostly along its walls when no electrical stimulation was provided.

In contrast, when we stimulated the anterior site in the MFB region as the subject entered the virtual cage indicated as a black circle on the right plot, it repeatedly entered the virtual cage, mostly staying around the area.

Similarly, when we stimulated the posterior site in the MFB region of the subject as it entered the virtual cage, it produced the same behavior as above, staying around

the virtual cage.

3.3. Behavioral analysis

This section shows the distance metric d. In figures 7 and 8, the temporal progression of the distance metric d through the trails is shown in line plots.

Below, bar plots compare the frame counts where $d < 0.1$ [m], i.e., the rat was attracted to remain within the virtual cage. The counts during anterior and posterior stimulation were normalized against those in the null condition.

Positive group Line plots in Figure 7 show that the subjects C and F stayed near the virtual cage throughout the sessions when stimulated either in the anterior or posterior site. The rats' behavior was remarkably different from the null condition when it just went across the virtual cage. This preference was observed both in POW 1 and 2 sessions.

Subject I, on the other hand, left the virtual cage incidentally after staying around it for 30 – 70 s, and failed to find the stimulation area in the POW 1 session. Interestingly, it stayed in the virtual cage in the POW 2 session.

The bar plots on Figure 7 indicate that all three subjects preferred staying in the virtual cage. Regardless of stimulated sites and POW session, the counts of attracted frames obviously increased with stimulation.

Negative group The subjects in the negative group had no tendency to stay near the virtual cage despite electrical stimulation in the brain. Line plots in Figure 8 indicate that subjects and sessions but not the stimuli were responsible for changing the distance metric d.

Rats showed no remarkable difference in the counts of the attracted frames either, except for the subject A, which was less active in the POW 1 session, incidentally staying near the virtual cage when the anterior site was stimulated. Even if it yielded the highest count of attracted frames, the subject showed no response to the stimuli.

3.4. Demonstration

Our Rattractor system showed the best performance on subject C during the first POW session. Here, we demonstrate how the subject was attracted to the virtual cage when the cage was either still or moving.

Still virtual cage Figure 9 indicates the times that subject C stayed on a specific location divided by a 15×10 mesh.

On the null condition without stimuli, the rat showed an exploring behavior over the experimental field, yielding no remarkable focus points. In contrast, the rat preferred staying near the virtual cage (illustrated as a red circle) where it could earn reward stimuli in the MFB.

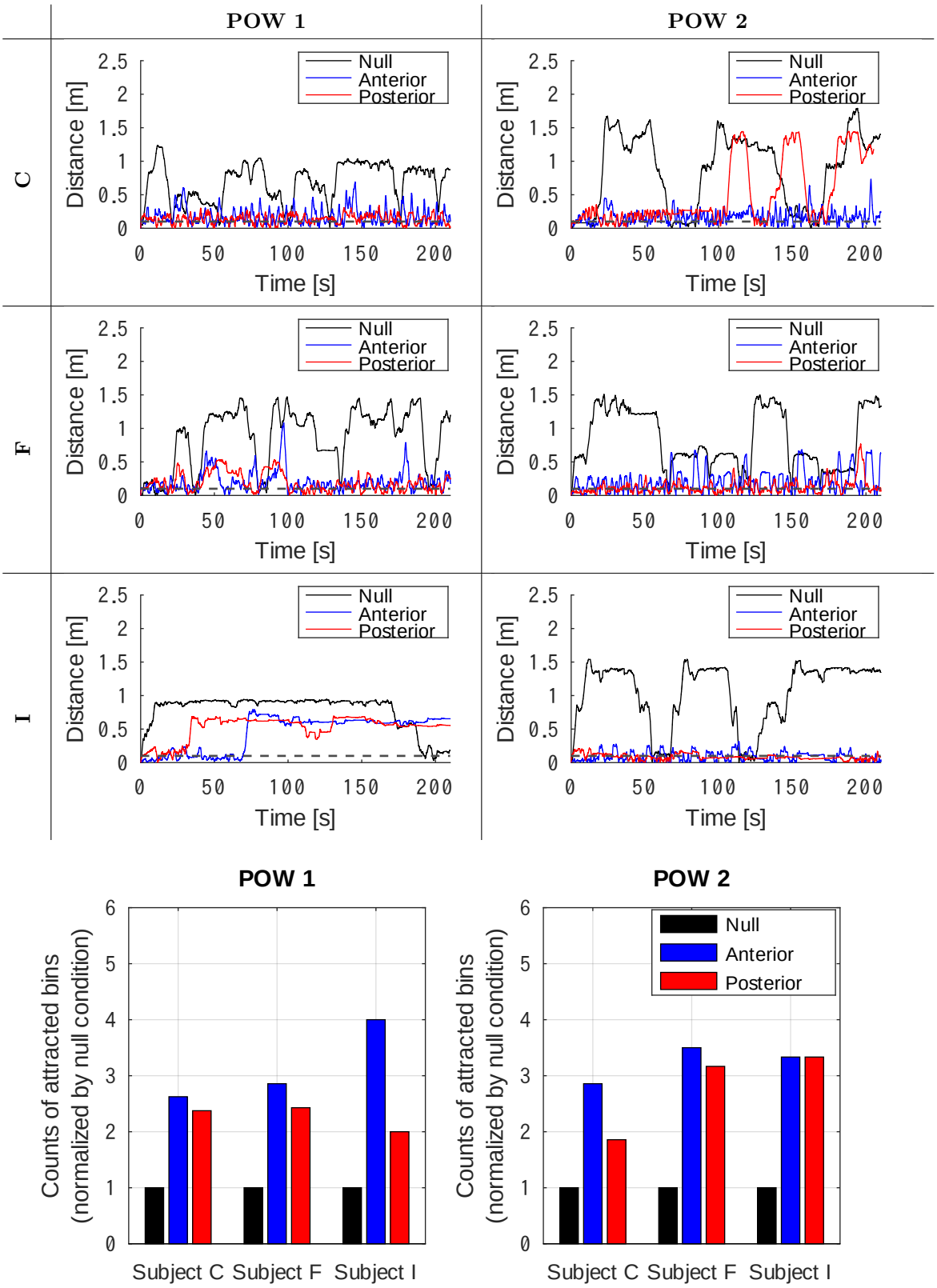

Figure 7. Line plots showing the temporal changes of the distance metric d throughout the sessions. The bar plots underneath compare the count of bins where the distance metric d stayed < 0.1 [m]. The analyses were conducted on POW 1 and 2 sessions for the subjects C, F, and I.

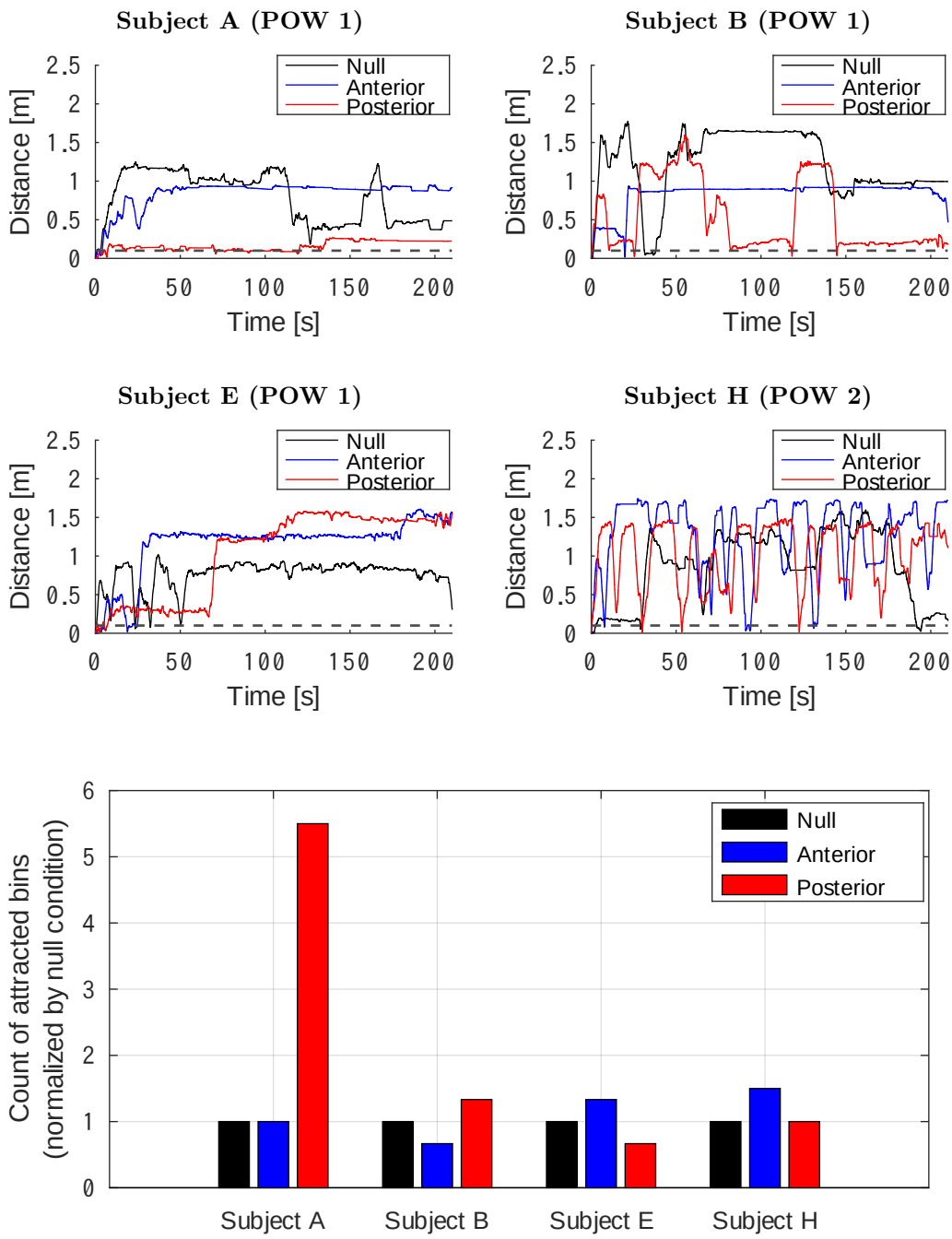

Figure 8. Line plots showing temporal changes in the distance metric d throughout sessions. Bar plots at the bottom comparing bin counts where the distance metric d was < 0.1 [m]. The analyses were conducted on subjects A, B, E, and H during the POW 1 session.

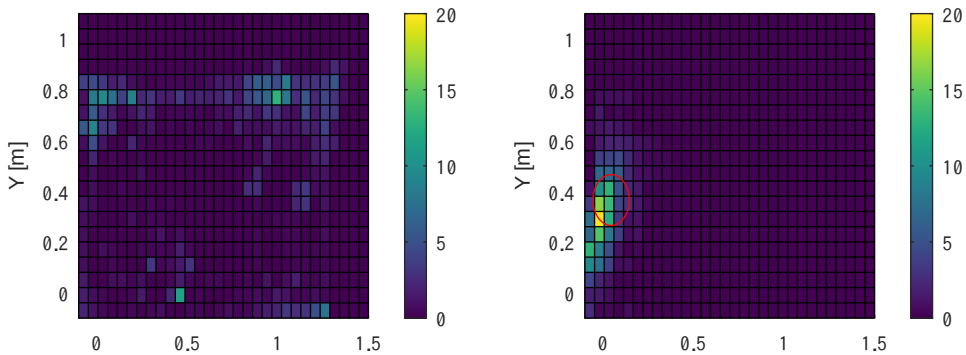

Figure 9. Histogram indicating the time s that the rat stayed on a specific location without stimulation (left), and in the virtual cage indicated by a red circle with stimuli in the MFB (right), respectively.

The subject repeatedly enter the virtual cage, occasionally stepped out, and then immediately head back to the area.

Moving virtual cage Figure 10 shows a trajectory of the subject C (light blue) and the virtual cage (dark red) which was manually moved. The virtual cage was initially set at the center of the field (X=0.75, Y=0.5), moved towards one corner (X=0.0, Y=1.0) at a constant velocity, and then moved towards the opposite corner (X=1.3, Y=0.0), before moving towards the approximate center of the field (X=0.75, Y=0.6).

The two plots below indicate the temporal changes in x and y coordinates, demonstrating that the rat followed the virtual cage.

4. Discussion & conclusion

This research aimed to provide a proof-of-concept of our "Rattractor" system, which can attract a rat into a virtual cage by providing electrical stimuli in the brain's reward region. Three out of seven rats yielded successful results after placing a set of two needle electrodes in the MFB (Figure 5), increasing the count of attracted bins compared to the null condition where no stimuli were applied (Figure 7).

Furthermore, subject C showed clear preference towards staying near the virtual cage set at arbitrary locations (Figure 9), which was consistent when the virtual cage moved slowly (Figure 10). Notably, the subject followed the virtual cage even across the center of the experimental field against their natural characteristics to avoid open spaces. A similar trend was observed in subjects F and I.

The performance of our system depended on electrode implantation. The three successful rats had their electrodes correctly located in the MFB region according to the subsequent histological analyses. In contrast, we did not find evidence of electrode tip locations within the MFB regions of the other four subjects which showed null or avoidant responses to the stimuli.

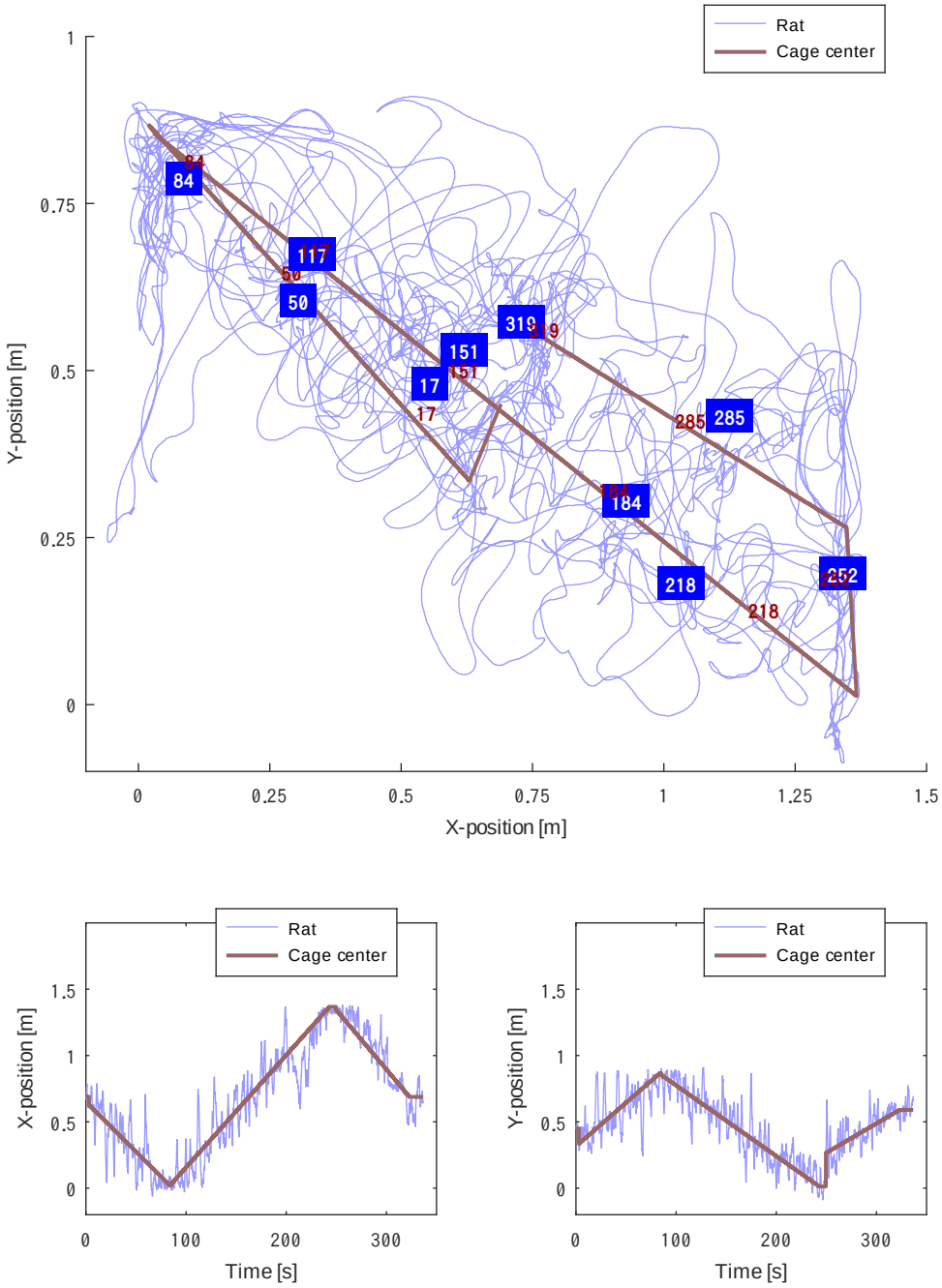

Figure 10. The plot above shows a two-dimensional trajectory of subject C (light blue) and the virtual cage (dark red), which was manually moved. The numbers show elapsed time s since the beginning of the session. The two plots below show temporal changes in x and y coordinates.

Stimulation in the MFB region could drive the brain reward circuit by evoking dopamine-like responses in the striatum[21]. Moreover, it could cause enforced learning to gain more rewards, motivating the subject to search for the virtual cage and stay in it. Thus, we aimed for the wide and long MFB region running parallel to the body axis to effectively induce rewards.

However, the correlation between successful behavior and correct electrode placement indicicates the relevance of electrode positiong for this system to work. We used a stereotaxic electrode manipulator to introduce the electrodes vertically into the brain from the surface to the target regions. However, possible failure in the perpendicular grab of the electrodes or individual differences in skull/brain form and size may have resulted in failure to reach the target region. We could not confirm the actual electrode position until histological analyses. Therefore, all behavioral experiments were conducted blind to electrodes positioning. Although we considered detecting the electrode tips with magnetic resonance (MR) imaging to avoid sacrificing the subjects, we concluded that the image accuracy would not be sufficient to determine the tip locations due to potential artifacts.

Stimulation conditions such as a current volume, frequency, and iteration number were determined according to previous work[6] and adjusted to obtain the best performance. These parameters had to be strong enough to induce changes in locomotion but weak enough to avoid abnormal cell activity resulting in epileptic responses. According to the present results, both demands were satisfied by the system.

Rattractor is essentially a closed-loop behavior-reward feedback system to restrict the animal behavior. Traditional approaches to restrict animal movement include physical cages, walls, and choker rings. These are simple and effective but not adaptable to various circumstances. Reward and punishment by a feeder allows the animals learn to stay within permitted areas. However, it requires training and is time consuming. In contrast, our rewarding system only requires the feeder to set a spatial region, which could be set arbitrarily and adaptively, towards which the animal will be attracted. In our system, the animal was motivated to stay within the virtual cage without physically restricting its behavior, allowing the animal to resist the lead if necessary. Furthermore, the feeder could terminate rewarding at any time and free the animal from this movement restriction.

These characteristics are well suited for grazing. For example, cattle and sheep could be led by the system within a farm without physical barriers, similarly to a shepherd dog chasing sheep. Although implanting electrodes into all animals in a group is unrealistic, performing the surgery in a few leaders might be enough to guide the rest.

In conclusion, we developed a “Rattractor” system to automatically attract a rat towards a virtual cage only when the system was activated, allowing free movement when deactivated, which was only effective as the system were activated. We have shown that our system could alter behavior of a rat and guide it towards arbitrary locations if we succeeded in placing the stimulation electrodes in MFB. Developing more precise surgery technique would increase the successful rate of the implants, and would lead to

evaluate the system with a larger number of subjects.

References

[1] P. Lopes, A. Ion, and P. Baudisch, "Impacto: Simulating P hysical I mpact by Combining Tactile Stimulation with Electrical Muscle Stimulation," in Proc. 28th ACM User Interface Software and Technology Symposium, Charlotte, NC, Nov. 2015, pp. 11–19.

[2] M. Pfeiffer, T. Dünte, S. Schneegass, F. Alt, and M. Rohs, "Cruise control for pedestrians: Controlling walking direction using electrical muscle stimulation," in Proc. CHI2015, Crossings, Seuol, Korea, Apr. 2015, pp. 2505–2514.

[3] S. Tatsuno, T. Hayakawa, and M. Ishikawa, "Supportive training system for sports skill acquisition based on electrical stimulation," in Prof. 2017 IEEE World Haptics COnference, Fürstenfeldbruck, Germany, Jun. 2017, pp. 466–471.

[4] D. Graupe, "EMG Pattern Analysis for Patient-Responsive Control of FES in Paraplegics for Walker-Supported Walking," IEEE Transactions on Biomedical Engineering, vol. 36, no. 7, Jul. 1989, pp. 711–719.

[5] Y. Nishimura, S. I. Perlmutter, and E. E. Fetz, "Restoration of upper limb movement via artificial corticospinal and musculospinal connections in a monkey with spinal cord injury," Frontiers in Neural Circuits, vol. 7, Apr. 2013, pp. 1–9.

[6] S. K. Talwar, S. Xu, E. S. Hawley, S. A. Weiss, K. A. Moxon, and J. K. Chapin, "Rat navigation guided by remote control," Nature, vol. 417, May 2002, pp. 37–38.

[7] T. Latif, and A. Bozkurt, "Roach Biobots Toward reliability and optimization of control," IEEE PULSE Magazine, vol. 27, Sep. 2017, pp. 2154–2287.

[8] A. Dirafzoon, T. Latif, F. Gong, M. Sichitiu, A. Blzkurt, and E. Lobaton, "Biobotic Motion and Behavior Analysis in Response to Directional Neurostimulation," in Proc. 42nd IEEE Intl. Conf. on Acoustics, Speech and Signal Processing, New Orleans, GA, USA, 2017, pp. 2457–2461.

[9] E. Whitmire, T. Latif, and A. Bozkurt, "Kinect-based System for Automated Control of Terrestrial Insect Biobots," in Proc. 35th Annu. Intl. Conf. IEEE EMBS, Osaka, Japan, 2013, pp. 1470–1473.

[10] N. Sudo, O. Fukayama, and K. Mabuchi, "Study on the deep brain stimulation to induce voluntary locomotion of a rat," in Proc. Society for Neuroscience Annual Meeting, Washington, DC, USA, Nov. 2017.

[11] O. Fukayama, N. Sudo, K. Mabuchi, and T. Isoyama, "Rattractor — Closed-loop Rat Attractor by Deep Brain Stimulation," in Proc. 40th Annu. Intl. Conf. IEEE EMBS, Honolulu, HI, USA, Jul. 2018.

[12] N. Sudo, O. Fukayama, K. Mabuchi, and T. Isoyama, "Modifying behavior of a rat during a Brain-Machine Interface experiment by deep brain stimulation," in Proc. Society for Neuroscience Annual Meeting, San Diego, CA, USA, Nov. 2018.

[13] D. L. Margules, and J. Olds, "Identical "Feeding" and "Rewarding" System in the Lateral Hypothalamus of Rats," Science, vol. 135, Sep. 1961, pp. 374–375.

[14] D. C. Castro, S. L. Cole, and K. C. Berridge, "Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry," Frontiers in Systems Neuroscience, vol. 9, Jun. 2015, pp. 1–17.

[15] Z. -B. You, Y. -Q. Chen, and R. A. Wise, "Dopamine and Glutamate Release in the Nucleus Accumbens and Ventral Tegmental Area of Rat Following Lateral Hypothalamic Self-Stimulation," Neuroscience, vol. 107, no. 4, 2001, pp. 629–639.

[16] J. Olds, and P. Milner, "Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain," J. Comparative and Physiological Psychology, vol. 47, no. 6, 1954, pp. 419–427.

[17] J. Olds, P. Travis, and C. Schwing, "Topographic Organization of Hypothalamic Self-Stimulation Functions," J. Comparative and Physiological Psychology, vol. 53, no. 1, 1960, pp. 23–32.

[18] G. Paxions, and C. Watson, The Rat Brain in Stereotaxic Coordinates Compact Third Edition. San Diego, CA: Academic Press, 1997.

[19] Nov. 2021. [Online]. Available: `https://opencv.org/`

[20] Nov. 2021. [Online]. Available: `http://comedi.org/`

[21] K. Yoshimi, S. Kumada, A. Weitemier, T. Jo, and M. Inoue, "Reward-Induced Phasic Dopamine Release in the Monkey Ventral Stiatum and Putamen," PLoS ONE, vol. 10, no. 6, e0130443, 2015.