Biochemical and Biophysical Research Communications 591 (2022) 20–25

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Delayed reinforcement hinders subsequent extinction

Yusuke Shibata [a, 1], Airi Yoshimoto [a, 1], Kotaro Yamashiro [a], Yuji Ikegaya [a, b, c], Nobuyoshi Matsumoto [a, b, *]

[a] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
[b] Institute for AI and Beyond, The University of Tokyo, Tokyo, 113-0033, Japan
[c] Center for Information and Neural Networks, National Institute of Information and Communications Technology, Suita City, Osaka, 565-0871, Japan

ARTICLE INFO

Article history:
Received 3 November 2021
Accepted 26 December 2021
Available online 28 December 2021

Keywords:
Medial forebrain bundle
Rat
Nose poke
Reward

ABSTRACT

In operant conditioning, animals associate their own behavior with a reinforcer, and the probability of the behavioral responses is increased. This form of learning is called reinforcement. In contrast, when the previously reinforced responses are no longer paired with a reinforcer, these responses are eventually extinguished. The effectiveness of reinforcement depends primarily on time intervals between reinforcers and responses, but it is not fully understood how the intervals affect subsequent extinction. To address this question, we performed electrical stimulation of the rat medial forebrain bundle (MFB), a part of the brain reward system, and an operant task in which the MFB was electrically stimulated 0.1 s (immediate condition) or 1 s (delayed condition) after the rat's nose was poked. During the first half of the task period (a reinforcement period), nose pokes were associated with MFB stimulation. In contrast, during the second half (an extinction period), we did not stimulate the MFB irrespective of nose pokes. We found that rats exhibited increased nose-poke behaviors during the reinforcement period under both conditions, whereas during the extinction period, nose pokes were more persistent in the delayed condition than in the immediate condition. The persistent responses in the extinction period were independent of responses in the reinforcement period. Therefore, reinforcement and extinction are driven by independent neural mechanisms.

1. Introduction

Associative learning is a process through which animals obtain information on links between external events and extract logical structures of entities as a consequence of repeated pairing of the events. In general, associative learning is categorized into two primary forms: classical Pavlovian conditioning and operant conditioning. In operant conditioning, animals learn to link their own behavior with either a reinforcer or a punisher and modify their responses; the responses are typically followed by an increase or decrease in the probability of behavioral responses, which is called reinforcement or punishment, respectively [1]. In contrast, when previously reinforced behavioral responses are no longer paired with a reinforcer, the responses become less frequent and even extinguish, a process that is called extinction [1].

The effectiveness of reinforcement and punishment depends on several factors: satiation/deprivation, immediacy, contingency, and size [2,3]. Among these factors, the principle of immediacy states that the more immediately reinforcement occurs after certain behavioral responses, the more effective reinforcement is in modifying such behavior [2–4]. While the effects of immediacy on reinforcement have been widely documented [5–7], it remains almost unknown whether immediacy has an impact on subsequent extinction.

To address this question, we made use of electrical stimulation of the medial forebrain bundle (MFB), a neural pathway containing complex ascending and descending fibers that course through and partially arise and terminate within various regions of the reward system, such as the ventral tegmental area, the nucleus accumbens, and the lateral hypothalamus [8–15]. Compared with food or water, electrical stimulation of the MFB is considered a reinforcer that elicits satiation/deprivation-free reinforcement [16–19]. Moreover, MFB stimulation allows us to precisely manipulate the time interval between behavioral responses and corresponding neural

* Corresponding author. Laboratory of Chemical Pharmacology Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan.
E-mail address: nobuyoshi@matsumoto.ac (N. Matsumoto).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2021.12.101

activation. We thus implanted stimulating electrodes into the rat MFB. We then employed an operant nose-poke behavioral test in which the rat MFB was electrically stimulated 0.1 s or 1 s after the nose poke (named the immediate condition or delayed condition, respectively). For each condition, we employed operant conditioning during the first half (0–30 min) of the experiment (*i.e.*, reinforcement period), whereas we did not provide any electrical stimulation to the MFB during the second half (30–60 min) (*i.e.*, extinction period) of the experiment. We quantified the nose-poke counts and locomotor activity to evaluate the effect of immediacy on reinforcement and extinction.

2. Materials and methods

2.1. Ethical approval

Animal experiments were performed with the approval of the Animal Experiment Ethics Committee at the University of Tokyo (approval number: P29-4) and according to the University of Tokyo guidelines for the care and use of laboratory animals. These experimental protocols were carried out in accordance with the Fundamental Guidelines for the Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions (Ministry of Education, Culture, Sports, Science and Technology, Notice No. 71 of 2006), the Standards for Breeding and Housing of and Pain Alleviation for Experimental Animals (Ministry of the Environment, Notice No. 88 of 2006) and the Guidelines on the Method of Animal Disposal (Prime Minister's Office, Notice No. 40 of 1995). All efforts were made to minimize animal suffering.

2.2. Animals

A total of thirty-one male 9-week-old or older Sprague–Dawley rats (Japan SLC, Shizuoka, Japan) were housed individually under conditions of controlled temperature and humidity (22 ± 1 °C, 55 ± 5%) and were maintained on a 12:12-h light/dark cycle (lights off from 7:00 to 19:00) with *ad libitum* access to food and water. Rats were habituated to an experimenter via daily handling for at least 2 d before experiments.

2.3. Preparation

To electrically stimulate the MFB, we designed a bundle of bipolar stainless-steel insulated wires (*i.e.*, a stimulation electrode, hereafter; TOG217-049c, Unique Medical, Tokyo, Japan) based on previous literature [20]. The stimulation electrode was composed of two insulated wires stuck together, whose tips were shifted by 500 μm in length. Each tip was bare for 100 μm. The other end of the electrode was soldered to a 2-pin connector protected with epoxy glue to prepare a stimulating electrode assembly.

2.4. Surgery

General anesthesia was induced in the rats and maintained with 2–3% and 1–2% isoflurane gas, with careful inspection of the animal's condition during the whole surgical procedure. Veterinary ointment was applied to the rats' eyes to prevent drying. The skin of the rats was sterilized with povidone iodine and 70% ethanol whenever an incision was made.

After anesthesia, each rat was mounted onto a stereotaxic apparatus (SR-6R-HT, Narishige, Tokyo, Japan) according to the general surgical procedure [21]. The scalp was then removed with a surgical knife. A circular craniotomy with a diameter of approximately 0.9 mm was performed using a high-speed dental drill. A stimulation electrode was stereotaxically implanted unilaterally into the MFB (2.0 mm posterior and 2.0 mm lateral to bregma). The stimulation electrode was carefully inserted into the rat's brain and lowered approximately 7.8 mm below the cortical surface. After implantation, the electrodes were secured to the skull using dental cement. Following surgery, each rat was allowed to recover from anesthesia and was housed individually in transparent plexiglass cages with free access to water and food. The animals were habituated to the experimenter again by handling for at least 5 d after surgery.

While our experimental protocols mandated the humane killing of animals if they exhibited any signs of pain, prominent lethargy, or discomfort, we did not observe such symptoms in any of the 31 rats used in this study.

2.5. Apparatus

A Skinner box (OP-3501, O'hara, Tokyo, Japan) with two nose-poke holes (20 mm in diameter) in a soundproof chamber was used. The box measured 40 cm in width, 30 cm in depth, and 40 cm in height. Nose pokes were detected by a photoelectric sensor in a hole and recorded using Arduino; note that only one 'active' hole was connected to the sensor, whereas the other was not.

2.6. Behavioral test

After the animals fully recovered from surgery and had become familiar with the apparatus, we performed electrophysiological experiments, including training (on Day 1) and test sessions (on Day 2). For each session, the stimulating electrode assembly was attached to a two-core cable. The cable was further connected to an isolator (A365, World Precision Instruments (WPI), FL, USA) and a stimulator (A310, WPI). During either session, each rat was allowed to freely explore the operant chamber for 1 h.

During the training session, each rat was rewarded by MFB stimulation whenever the rat poked its nose into the active hole. Rats that poked their noses into the active hole over 150 times during the training session were used during the subsequent test session (on the next day). The amplitude of MFB stimulation pulse currents was optimized during this training session (described below).

During the test session, rats were allowed to behave in the same way as during the training session, but they were rewarded during the first half (30 min) of the session (*i.e.*, reinforcement period), while they were unrewarded even though they poked their noses into either hole during the second half (*i.e.*, extinction period). In the reinforcement period (during the test session), 13 and 18 rats underwent the 'immediate' and 'delayed' conditions, respectively. Under immediate conditions, each rat received electrical stimulation of the MFB immediately (0.1 s) after their nose pokes. On the other hand, in the delayed condition, rats were electrically rewarded 1 s after nose pokes.

The number of nose pokes was counted by Arduino custom routines. Animal behavior was monitored using a web camera at 10–30 fps throughout the experiment.

Rectangular symmetrical biphasic electric currents were generated by a stimulator. Parameters for the electric currents were as follows: *amplitude* (for each of the positive and negative phases), 200–300 μA; *phase duration* (for each phase), 0.2 ms; *interphase interval*, 0 s; *interpulse interval* (time between onsets of a positive phase and the next), 10 ms (*i.e.*, *pulse frequency*, 100 Hz); and *burst duration*, 500 ms. The parameters except for the amplitude were determined by our preliminary study. During the training session (described above), the appropriate amplitude of the electrical stimulation was set as the value that met the criteria of allowing rats to poke their noses more than 150 times without a convulsive

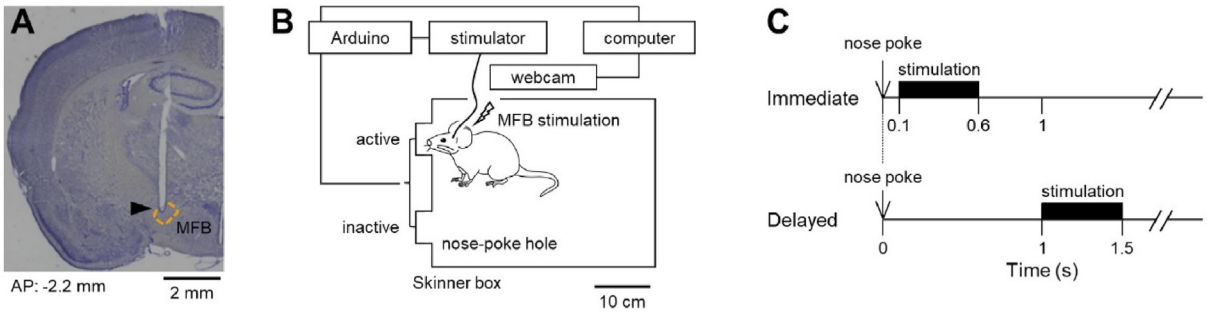

Fig. 1. Overview of the experiment.
A, A representative image of a Nissl-stained coronal section. The tip of the track of the stimulation electrode is indicated by *black* triangles. The track of the electrode tip was found in the MFB (*yellow*). ***B***, A diagram of the experimental setup. Once a rat poked its nose into an active hole, direct electrical stimulation was delivered to the rat's MFB as a reward. ***C***, Experimental paradigms for immediate and delayed conditions. A rat received 0.5 s of electrical stimulation 0.1 s after a nose poke under immediate conditions. Another rat was rewarded 1 s after the nose poke. *Abbreviation*: MFB, medial forebrain bundle. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

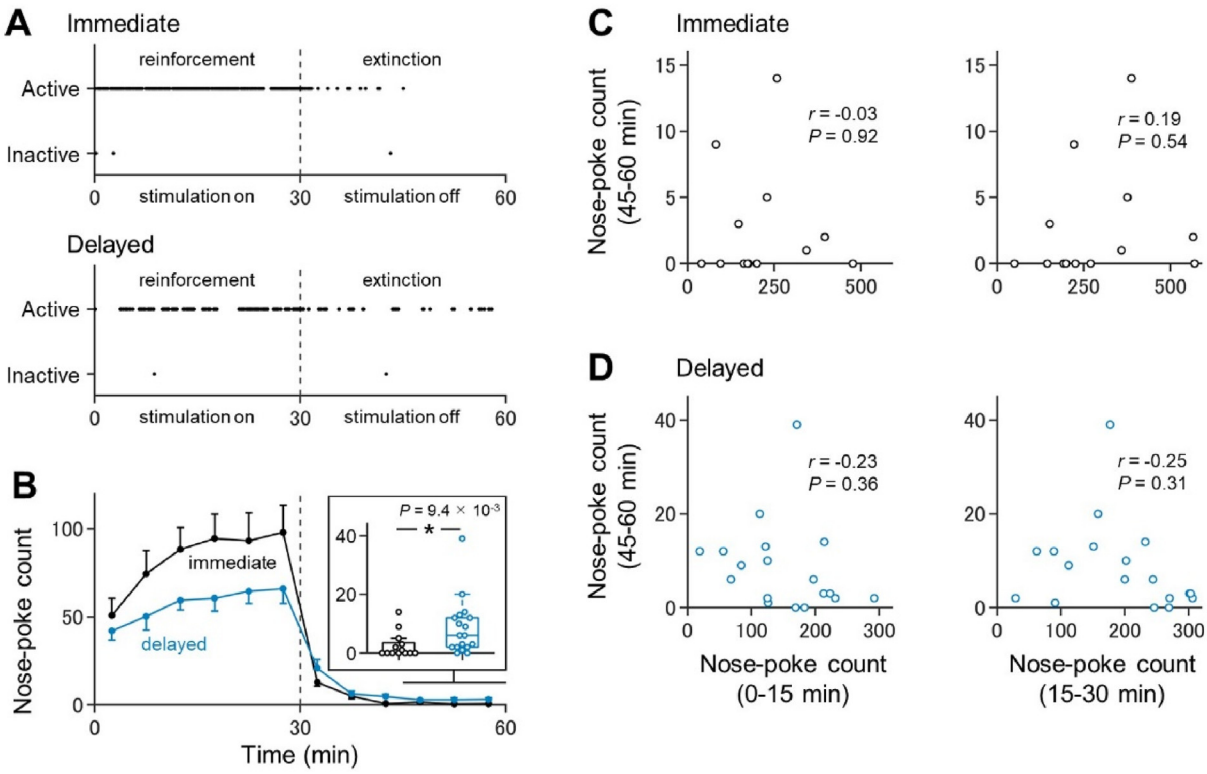

Fig. 2. Persistent nose-poke behavior during the extinction period.
A, Representative raster plots indicating nose pokes into active and inactive holes in the immediate (*top*) and delayed (*bottom*) conditions. Note that the first and second halves of the experiment are named the reinforcement and extinction periods, respectively. ***B***, Time course of nose-poke counts of rats that underwent the immediate (*black*) and delayed (*blue*) conditions. In particular, during the fourth quarter of the experiment, the nose-poke counts were significantly larger in the delayed condition than in the immediate condition. ***C***, Nose-poke counts during the fourth quarter were not significantly correlated with those during the first quarter (*left*) or the second quarter (*right*) for the immediate condition. ***D***, The same as *C*, but for the delayed condition. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fit, confirming that the stimulation current did not negatively affect animal behavior [22]. The amplitude varied among animals, but once it was optimized for a given animal during the training session, it was constant throughout the experiment (including the training and test sessions). After the appropriate parameters of the currents were fixed, electrical stimulation was delivered to the rat's MFB as a reward 0.1 s or 1 s after a nose poke (into the active hole) during the reinforcement (not extinction) period of the test session. For both sessions, the refractory period of the burst was 100 ms; that is, a rat was never received MFB stimulation until after 100 ms from the previous stimulation.

2.7. Histology

After recordings, rats were anesthetized with an overdose of urethane, transcardially perfused with 0.01 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde (PFA) in 0.01 M PBS, and decapitated. After decapitation, the heads were stored at 4 °C for 4 h, and the brains were carefully removed. The brains were soaked in 20% sucrose (in 4% PFA) overnight for post-fixation and cryoprotection and coronally sectioned at a thickness of 50 μm using a cryostat (CM3050S, Leica Biosystems, Wetzlar, Germany). Serial slices were then mounted on glass slides and

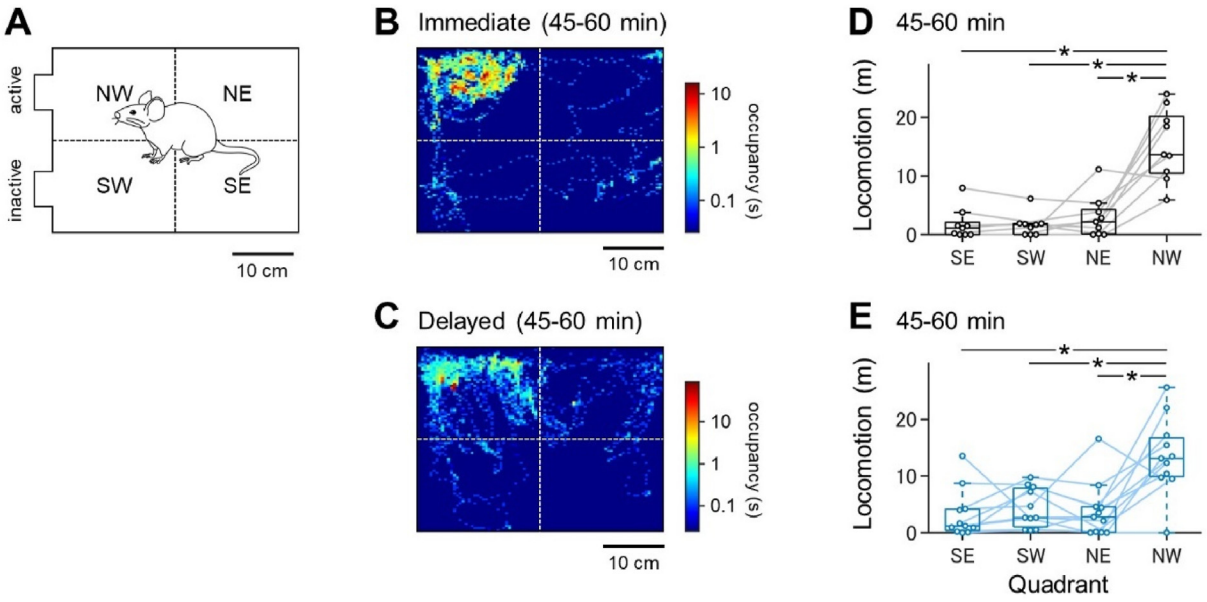

Fig. 3. Locomotor activity in subareas of the chamber in both the immediate and delayed conditions.
A, The operant chamber is divided into four quadrants (*i.e.*, NW, NE, SE, and SW). ***B***, A representative pseudocolor map indicating the time for which a rat occupied the corresponding place during the fourth quarter of the experiment under immediate conditions. Hot and cold colors indicate long and short times, respectively. ***C***, The same as *B*, but for the delayed condition. ***D***, The total amount of the trajectory taken by rats in each quadrant during the fourth quarter of the experiment under immediate conditions. ***E***, The same as *D*, but for the delayed condition. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stained with cresyl violet. Cresyl violet staining was performed based on a previously described procedure [23,24]. Briefly, the slices were rinsed in water, ethanol, and xylene; counterstained with cresyl violet; and coverslipped with a mounting agent (PARAmount-D, FALMA, Tokyo, Japan). The positions of the stimulating electrode tips were confirmed by identifying a track of the electrode. Data were excluded from the subsequent analysis if the tip position was outside the MFB. Cresyl violet-stained images were acquired using a phase-contrast microscope (BZ-X710, Keyence, Osaka, Japan).

2.8. Data analysis

All data analyses were performed using custom-made MATLAB (MathWorks, MA, USA) and Python routines. The summarized data are reported as the mean ± the standard error of the mean (SEM). The null hypothesis was statistically rejected when $P < 0.05$, unless otherwise specified. When multiple pairwise comparisons were required, the original P value was compared with the adjusted significance level based on Bonferroni correction.

DeepLabCut, a markerless tracking system, was utilized to track the rats' moment-to-moment positions as previously described [25,26]. Paths traveled by rats and rats' locomotion were quantified based on the x and y coordinates of their heads.

3. Results

We implanted a stimulation electrode into the MFB of rats (Fig. 1A) and allowed them to freely poke their noses in the operant chamber (Fig. 1B). We delivered electrical stimulation to the MFB when the rats poked their noses into the active hole. Some rats were electrically rewarded immediately (0.1 s) after a nose poke, whereas the others were rewarded 1 s after their nose pokes (Fig. 1C).

Under both immediate and delayed conditions, the rats frequently poked their noses into the active hole during the reinforcement period (Fig. 2A). Unexpectedly, the rats seemed to persistently exhibit nose-poke behavior during the extinction period under the delayed condition compared with their behavior in the immediate condition (Fig. 2A and B). In particular, even 15–30 min after MFB stimulation was terminated (*i.e.*, 45–60 min from the beginning of the experiment), the nose-poke counts were significantly higher in the delayed condition than in the immediate condition ($P = 9.4 \times 10^{-3}$, 10,000 surrogates, permutation test; Fig. 2B inset). The nose-poke counts in the fourth quarter (*i.e.*, 45–60 min) of the experiment were not significantly correlated with those in the first or second quarters under either the immediate or delayed condition (Fig. 2C and D), suggesting that the reinforcement and extinction learnings are independent.

We further quantified each rat's trajectory and occupancy in the chamber during the fourth quarter and found that rats were likely to stay in the NW quadrant (*i.e.*, the quadrant in the chamber closest to the active hole) under both immediate and delayed conditions (Fig. 3A–C). The paths traveled by rats in the NW quadrant were significantly larger than those in any of the other three quadrants not only under the immediate condition ($P = 5.4 \times 10^{-4}$, $t_8 = 5.5$, $n = 9$ rats (for SE *vs.* NW), $P = 3.1 \times 10^{-4}$, $t_8 = 6.0$, $n = 9$ rats (for SW *vs.* NW), $P = 1.4 \times 10^{-3}$, $t_8 = 4.8$, $n = 9$ rats (for NE *vs.* NW), paired t-test, note that the significance level was adjusted to 8.3×10^{-3}; Fig. 3D) but also under the delayed condition ($P = 5.7 \times 10^{-3}$, $t_{10} = 3.5$, $n = 11$ rats (for SE *vs.* NW), $P = 2.5 \times 10^{-3}$, $t_{10} = 4.0$, $n = 11$ rats (for SW *vs.* NW), $P = 3.8 \times 10^{-3}$, $t_{10} = 3.7$, $n = 11$ rats (for NE *vs.* NW), paired t-test with Bonferroni correction; that is, the significance level for the paths traveled was adjusted to 8.3×10^{-3}; Fig. 3E). The total length of paths in the quadrant NW was not significantly different between the immediate and delayed conditions ($P = 0.56$, $t_{18} = 0.60$, $n = 20$ rats, Student's t-test).

4. Discussion

Using electrical stimulation of the MFB, we reinforced the rat's nose-poke behavior and found that the nose-poke behavior

persisted even after the rewarding stimulation ceased. The persistent behavior during the extinction session was independent of the nose pokes during the reinforcement session. Moreover, the rats hovered around the active nose-poke hole in both the immediate and delayed conditions, but the locomotion around the active hole was almost the same between the two conditions.

Consistent with a previous study [5], we found that a 1-s delay between the rat's nose poke and reward delivery significantly impeded the acquisition of self-stimulation behavior (Fig. 2B). However, nose-poke behavior persistently continued even after the association between the action and delayed rewards was terminated. This persistent nose-poke behavior was not affected by the previous conditioning (Fig. 2C and D). Moreover, the paths traveled by rats during the second half of the extinction session were not significantly different between the immediate and delayed conditions (Fig. 3D and E), suggesting that the delayed rewards did not impair motor activity. These results indicate that delayed rewards may prevent animals from extinguishing the association between their actions and rewards, *per se*, without negatively affecting motor activity.

Compared with food and water [27], direct electrical stimulation of the reward system is suitable to quantitatively evaluate the persistent effect of rewards on a fine time scale. Classically, food or water has been used as a reward in the operant conditioning of animals [28], but this type of conditioning should be terminated sooner once starvation or thirst is relieved. In other words, the value of food or water is likely to be gradually degraded, whereas the direct stimulation of the MFB would continuously provide a stable reward value. Moreover, food or water takes a longer time to exert an effect on neural activity as a reward than direct intracranial stimulation because the time spent in consumption varies between animals. Electrical stimulation excites neurons directly and immediately and enables us to precisely assess the effects of reinforcer delays on operant conditioning. As MFB stimulation has an impact on not only the reward system [29] but also memory- or emotion-related regions, such as the hippocampus [30], the prefrontal cortex [29], and the amygdala [30], it is difficult to determine the brain regions responsible for the sustained effect of delayed reinforcement during extinction. Further photometric methods combined with direct MFB stimulation could reveal the neural mechanism underlying the reinforcement phenomenon in this study and characterize new aspects of operant conditioning.

Declaration of competing interest

The authors declare that they have no conflicts of interest with respect to this research.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by JST ERATO (JPMJER1801), Institute for AI and Beyond of the University of Tokyo, and JSPS Grants-in-Aid for Scientific Research (18H05525, 20K15926).

References

[1] J.E.R. Staddon, D.T. Cerutti, Operant conditioning, Annu. Rev. Psychol. 54 (2003) 115–144, https://doi.org/10.1146/annurev.psych.54.101601.145124.
[2] L.K. Miller, Principles of Everyday Behavior Analysis, fourth ed., Wadsworth Publishing, 2005.
[3] R.G. Miltenberger, Behavior Modification : Principles and Procedures, sixth ed., Cengage Learning, 2015.
[4] S.F. Jablonsky, D.L. DeVries, Operant conditioning principles extrapolated to the theory of management, Organ. Behav. Hum. Perform. 7 (1972) 340–358, https://doi.org/10.1016/0030-5073(72)90021-9.
[5] J. Black, J.D. Belluzzi, L. Stein, Reinforcement delay of one second severely impairs acquisition of brain self-stimulation, Brain Res. 359 (1985) 113–119, https://doi.org/10.1016/0006-8993(85)91418-0.
[6] J.B. Richards, S.H. Mitchell, H. de Wit, L.S. Seiden, Determination of discount functions in rats with an adjusting-amount procedure, J. Exp. Anal. Behav. 67 (1997) 353–366, https://doi.org/10.1901/jeab.1997.67-353.
[7] H. Yin, Y. Wang, X. Zhang, P. Li, Feedback delay impaired reinforcement learning: principal components analysis of Reward Positivity, Neurosci. Lett. 685 (2018) 179–184, https://doi.org/10.1016/j.neulet.2018.08.039.
[8] R. Nieuwenhuys, L.M.G. Geeraedts, J.G. Veening, The medial forebrain bundle of the rat. I. General introduction, J. Comp. Neurol. 206 (1982) 49–81, https://doi.org/10.1002/cne.902060106.
[9] J.G. Veening, L.W. Swanson, W.M. Cowan, R. Nieuwenhuys, L.M.G. Geeraedts, The medial forebrain bundle of the rat. II. An autoradiographic study of the topography of the major descending and ascending components, J. Comp. Neurol. 206 (1982) 82–108, https://doi.org/10.1002/cne.902060107.
[10] L.M.G. Geeraedts, R. Nieuwenhuys, J.G. Veening, Medial forebrain bundle of the rat: III. Cytoarchitecture of the rostral (telencephalic) part of the medial forebrain bundle bed nucleus, J. Comp. Neurol. 294 (1990) 507–536, https://doi.org/10.1002/cne.902940403.
[11] A.J. Fenoy, J. Quevedo, J.C. Soares, Deep brain stimulation of the “medial forebrain bundle”: a strategy to modulate the reward system and manage treatment-resistant depression, Mol. Psychiatr. (2021), https://doi.org/10.1038/s41380-021-01100-6.
[12] S.S. Negus, L.L. Miller, Intracranial self-stimulation to evaluate abuse potential of drugs, Pharmacol. Rev. 66 (2014) 869–917, https://doi.org/10.1124/pr.112.007419.
[13] M.D. Döbrössy, C. Ramanathan, D. Ashouri Vajari, Y. Tong, T. Schlaepfer, V.A. Coenen, Neuromodulation in Psychiatric disorders: experimental and Clinical evidence for reward and motivation network Deep Brain Stimulation: focus on the medial forebrain bundle, Eur. J. Neurosci. 53 (2021) 89–113, https://doi.org/10.1111/ejn.14975.
[14] R.A. Wise, R.A. McDevitt, Drive and reinforcement circuitry in the brain: origins, neurotransmitters, and projection fields, Neuropsychopharmacology 43 (2018) 680–689, https://doi.org/10.1038/npp.2017.228.
[15] H. Takagi, S. Shiosaka, M. Tohyama, E. Senba, M. Sakanaka, Ascending components of the medial forebrain bundle from the lower brain stem in the rat, with special reference to raphe and catecholamine cell groups. A study by the HRP method, Brain Res. 193 (1980) 315–337, https://doi.org/10.1016/0006-8993(80)90168-7.
[16] S.K. Talwar, S. Xu, E.S. Hawley, S.A. Weiss, K.A. Moxon, J.K. Chapin, Rat navigation guided by remote control, Nature 417 (2002) 37–38, https://doi.org/10.1038/417037a.
[17] W.A. Carlezon, E.H. Chartoff, Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation, Nat. Protoc. 2 (2007) 2987–2995, https://doi.org/10.1038/nprot.2007.441.
[18] M.S. Todtenkopf, A. Parsegian, A. Naydenov, R.L. Neve, C. Konradi, W.A. Carlezon, Brain reward regulated by AMPA receptor subunits in nucleus accumbens shell, J. Neurosci. 26 (2006) 11665–11669, https://doi.org/10.1523/JNEUROSCI.3070-06.2006.
[19] G. Hunt, I. McGregor, Rewarding brain stimulation induces only sparse Fos-like immunoreactivity in dopaminergic neurons, Neuroscience 83 (1998) 501–515, https://doi.org/10.1016/S0306-4522(97)00409-0.
[20] K. Badstübner, M. Stubbe, T. Kröger, E. Mix, J. Gimsa, Impedance detection of the electrical resistivity of the wound tissue around deep brain stimulation electrodes permits registration of the encapsulation process in a rat model, J. Electr. Bioimpedance. 8 (2017) 11–24, https://doi.org/10.5617/jeb.4086.
[21] J. Maciaczyk, U.D. Kahlert, M. Döbrössy, G. Nikkhah, Stereotactic surgery in rats, in: M. Janowski (Ed.), Exp. Neurosurg. Anim. Model., Humana Press, New York, New York, USA, 2016, pp. 31–54, https://doi.org/10.1007/978-1-4939-3730-1_3.
[22] C. Kong, J. Shin, C.-S. Koh, J. Lee, M.-S. Yoon, Y.K. Cho, S. Kim, S.B. Jun, H.H. Jung, J.W. Chang, Optimization of medial forebrain bundle stimulation parameters for operant conditioning of rats, Stereotact. Funct. Neurosurg. 97 (2019) 1–9, https://doi.org/10.1159/000497151.
[23] A. Yoshimoto, K. Yamashiro, T. Suzuki, Y. Ikegaya, N. Matsumoto, Ramelteon modulates gamma oscillations in the rat primary motor cortex during non-REM sleep, J. Pharmacol. Sci. 145 (2021) 97–104, https://doi.org/10.1016/j.jphs.2020.11.006.
[24] A. Yoshimoto, K. Yamashiro, Y. Ikegaya, N. Matsumoto, Acute ramelteon treatment maintains the cardiac rhythms of rats during non-REM sleep, Biol. Pharm. Bull. 44 (2021) 789–797, https://doi.org/10.1248/bpb.b20-00932.
[25] A. Mathis, P. Mamidanna, K.M. Cury, T. Abe, V.N. Murthy, M.W. Mathis, M. Bethge, DeepLabCut: markerless pose estimation of user-defined body parts with deep learning, Nat. Neurosci. 21 (2018) 1281–1289, https://doi.org/10.1038/s41593-018-0209-y.

[26] K. Yamashiro, M. Aoki, N. Matsumoto, Y. Ikegaya, Polyherbal formulation enhancing cerebral slow waves in sleeping rats, Biol. Pharm. Bull. 43 (2020) 1356–1360, https://doi.org/10.1248/bpb.b20-00285.
[27] S. Kobayashi, W. Schultz, Influence of reward delays on responses of dopamine neurons, J. Neurosci. 28 (2008) 7837–7846, https://doi.org/10.1523/JNEUROSCI.1600-08.2008.
[28] K.E. Renner, Delay of reinforcement: a historical review, Psychol. Bull. 61 (1964) 341–361, https://doi.org/10.1037/h0048335.
[29] K. Krautwald, H.-K. Min, K.H. Lee, F. Angenstein, Synchronized electrical stimulation of the rat medial forebrain bundle and perforant pathway generates an additive BOLD response in the nucleus accumbens and prefrontal cortex, Neuroimage 77 (2013) 14–25, https://doi.org/10.1016/j.neuroimage.2013.03.046.
[30] E. Kádár, E.V. Varela, L. Aldavert-Vera, G. Huguet, I. Morgado-Bernal, P. Segura-Torres, Arc protein expression after unilateral intracranial self-stimulation of the medial forebrain bundle is upregulated in specific nuclei of memory-related areas, BMC Neurosci. 19 (2018) 48, https://doi.org/10.1186/s12868-018-0449-5.