Doctoral Dissertation

Effects of different reinforcement schedules on neuronal operant conditioning

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Abstract

Neuronal operant conditioning, in which rewards are given for modulations of neuronal firing independent of overt behaviors, is a core process for better operation of brain-machine interfaces (BMIs). However, there have been few systematic investigations of the methodology for operant conditioning of neuronal activity. In particular, few studies have investigated the role of reinforcement schedules in neuronal operant conditioning, although it has been postulated that different reinforcement schedules significantly impact the learning in behavioral operant conditioning. To test the effects of different reinforcement schedules, we trained neuronal activity in the motor cortex using fixed ratio (FR) and variable ratio (VR) schedules in rats. During the neuronal operant conditioning, a single neuron was recruited as a target, and the number of spikes of the target neuron was counted. Rats were rewarded whenever the number of spikes reached a predetermined threshold, and thus the rats could get rewards more frequently if they enhance the target neuron's firing rates. The rats in the FR schedule successfully learned to enhance their target neurons' firing rates to obtain rewards more frequently. On the contrary, the rats reinforced by the VR schedule exhibited no such learning until the last day of the conditioning. In addition, the neuronal data analyzed off-line demonstrated that specific neuronal activity such as peak firing rates around reward delivery was selectively appeared in the FR schedule. These results suggest that the reinforcement schedules differentially affect the learning in neuronal operant conditioning and cause various changes in activity of individual neurons. We expect these findings to contribute the research on the development of clinically significant and highly reliable BMIs using the plastic characteristics of the brain.

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Chapter 1. Introduction

1.1. Neuronal operant conditioning as a core mechanism of brain-machine interface

When we require learning of volitional enhancement of a certain behavior, operant conditioning (Skinner, 1974; Reynolds, 1975) should be the first choice. The voluntary behavior immediately followed by reward, i.e., having contingency of reward, soon becomes more frequent, and humans and animals volitionally conduct the behavior more frequently to get more reward. Based on such methodology, an intriguing method of learning of volitional enhancement in neuronal firing has been developed and called "neuronal operant conditioning", in which rewards are given for modulations of neuronal firing independent of overt behaviors (Fig. 1A). Since Olds (1965) and Fetz (1969) published their pioneering research, conditioned enhancement of neuronal firing has been frequently reported in animals and humans. In particular, Fetz and collaborators (Fetz, 1969; Fetz and Finocchio, 1971; Fetz and Baker, 1973) had established the methodology of neuronal operant conditioning and reported that monkeys could control firing rates of individual neurons in the motor cortex.

Researches investigating neuronal operant conditioning are steadily becoming more prolific (Arduin et al., 2013; Engelhard et al., 2013; Hwang et al., 2013; Koralek et al., 2012) given its relation to brain-machine interface (BMI). As Fetz (2007) suggested, the basic paradigm for neuronal operant conditioning is essentially identical to the one for BMI. BMI is for neuroprosthetic control of external devices by neuronal activity instead of behavior (Berger et al., 2008; Hatsopoulos & Donoghue, 2010; Nicolelis & Lebedev, 2009; Andersen et al., 2010; Green & Kalaska, 2011). BMI is unquestionably an innovative technology that is undergoing extensive and rapid progress (Birbaumer, 2006; Velliste et al., 2008; Moritz et al., 2008; Moran, 2010; Green and Kalaska, 2011). It enables neuroprosthetic control of external devices by brain activity instead of

bodily movements (Chapin et al., 1999; Donoghue et al., 2007; Nicolelis, 2011; Lebedev, 2014).

1.2. Limitations in development of an effective BMI

Despite the development of BMI is promising (Lebedev & Nicolelis, 2006), currently available BMI is still limited in terms of accuracy and the facility with which they can be controlled (Sakurai and Song, 2016). At the present time, the information coding in the brain is not completely understood (Rieke and Warland, 1999), and thus the ability to accurately decode and use the neural activity as the source of BMI is not currently possible. In addition, in most BMI experiments based on the decoding approach, conversion of neuronal signals is aided by appropriate transform algorithms to generate the adequate control parameters. The conversion parameters, however, obtained for one set of trials provided increasingly poor predictions of future responses, indicating a source of drift over tens of minutes (Sakurai and Song, 2016).

Although improvements in such technical factors affecting BMI performance are actively being pursued (Ethier et al., 2012; Lebedev and Nicolelis, 2011), some previous studies (Andersen et al., 2010; Nicolelis and Lebedev, 2009) have also emphasized that improvements in technical factors alone cannot solve all problems that hinder the development of an ideal BMI, i.e., a system controlling external neuroprosthetic devices without any special training. Besides the technical improvements, increased knowledge of brain mechanisms is absolutely needed (Baranauskas, 2014; Mandonnet and Duffau, 2014; Sakurai, 2014; Velliste et al., 2014). In particular, knowing how neuronal activity changes in the face of network plasticity is essential (Sakurai, 2014), since accurate device control by BMI inevitably requires neuronal activity to be volitionally modulated (Sakurai et al., 2014).

One of the most significant factors limiting the performance of BMI is plastic changes in neuronal activity induced by using BMI itself (Zacksenhouse et al., 2007; Ganguly et al., 2011). Operating machines with BMI can be considered as achieving some type of goal. If that goal is successfully

achieved, or if the machine can be operated skillfully, then this functions as a reward and will enhance the plastic change of neural activity through reinforcement feedback. In other words, BMI changes the brain activity itself to enable the acquisition of a reward by directly operating a machine without using the physical body (Sakurai et al., 2014). Those kinds of changes in the process of brain activity induced by reward are nothing but neuronal operant conditioning, indicating that investigation of the paradigm and methodology of neuronal operant conditioning is absolutely needed for the development of effective BMI (Fetz, 2007).

1.3. Reinforcement schedule, yet uninvestigated feature of neuronal operant conditioning

However, there have been few systematic investigations of the methodology for operant conditioning of neuronal activity. In particular, it has not been clearly demonstrated how reinforcement schedules affect learning in neuronal operant conditioning for volitional modulation of neuronal activity, although they are known to have a significant effect on an animal's operant responses in behavioral studies (Reynolds, 1975).

In the present study, we focused on the effects of reinforcement schedules applied to single neurons of the motor cortex during neuronal operant conditioning in rats. We used two basic—yet different—schedules of reinforcement: fixed ratio (FR) and variable ratio (VR). Both schedules have been shown to increase the probability of occurrence of behavioral response in many animal species and humans, with the former causing stable and moderate rates of response, and the latter causing rapid and high rates (Reynolds, 1975) (Fig. 1B). We tested whether these effects of FR and VR schedules of reinforcement on behavioral responses exist in operant conditioning of neuronal activity.

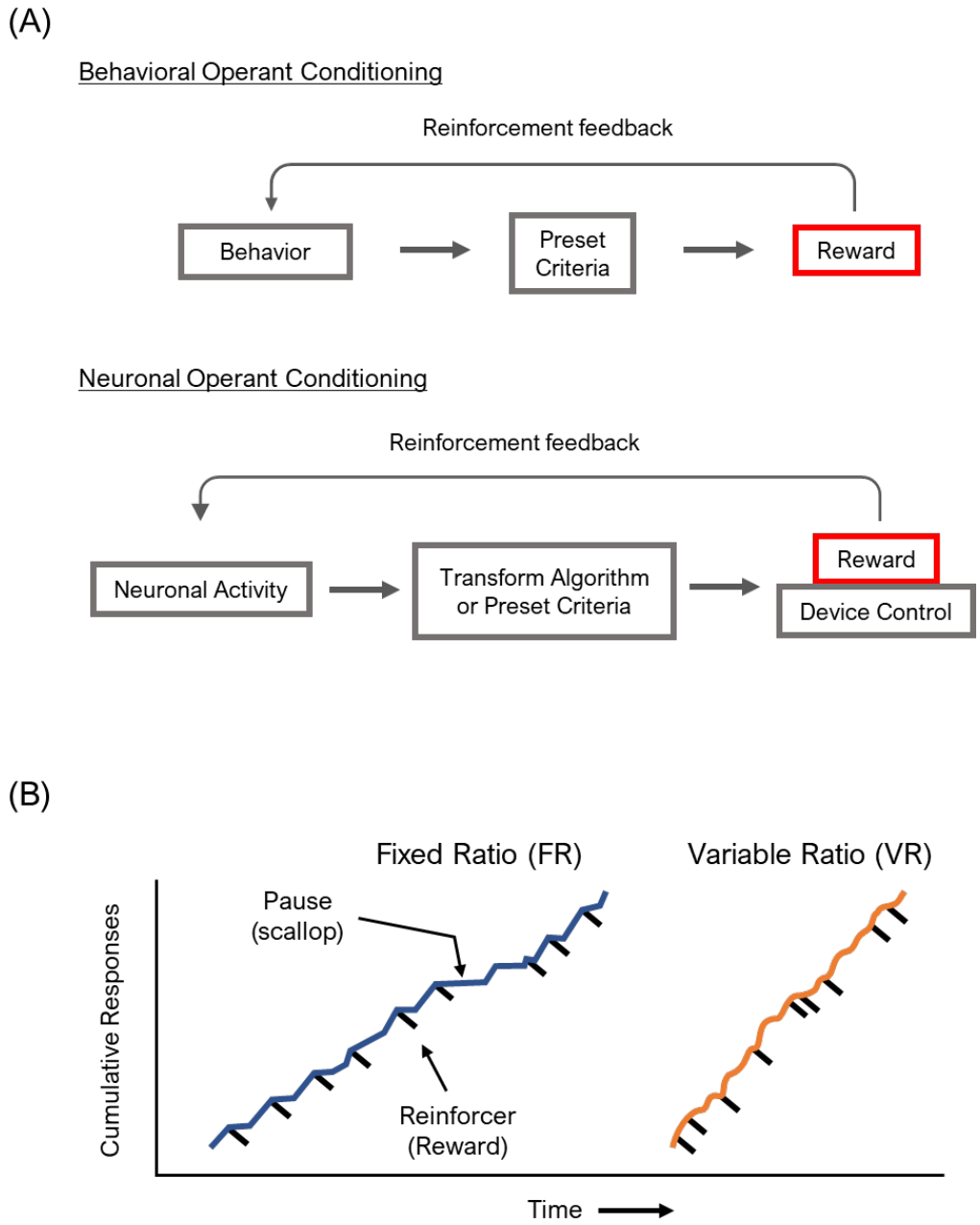

Fig. 1. Schematic diagram of the operant conditioning and reinforcement schedules

(A) Basic components of the neuronal operant conditioning and the behavioral operant conditioning paradigm were shown. The voluntary behavior satisfying preset criteria immediately followed by reward becomes more frequent in behavioral operant conditioning. In a very similar manner, the volitional modulation in the neuronal activity becomes more frequent when the modulation is properly reinforced in neuronal operant conditioning. (B) Both schedules generally increase the probability of occurrence of behavioral response in many animal species and humans. FR schedule causes stable and moderate rates, and the VR schedule does rapid and high response rates. On the contrary to the VR schedule, pause after a reward delivery is typically observed in the FR schedule.

Chapter 2. Materials and Methods

2.1. Animals

Nine male albino Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan), weighing 410–470 g, were used as subjects. The animals were individually housed in a 25 × 15 × 20 cm cages under a light and dark cycle (lights on at 08:00, lights off at 21:00). The animals' diets were restricted so that their weights were maintained to levels as stable as possible throughout the experiment. They had ad libitum access to water in their home cages except during the behavioral and neuronal operant conditioning periods, in which access to water was completely restricted and they could get water only as reward in the tasks. On the contrary, they were allowed to access to diets freely during the conditioning periods. The total amount of water taken through the reward a day was about 2-2.5% of the rats' weight. With that amount of water, all rats maintained their weights through the experiment. All experiments were performed in accordance with guidelines for animal experiments at Doshisha University.

2.2. Apparatus

Whole training sessions were conducted in an operant box (22 × 49 × 45 cm) (O'Hara & Co., Tokyo, Japan). To avoid possible distractions such as external light, noisy sounds or electrical noise, the operant box was installed in a shield box (Japan Shield Enclosure, Osaka, Japan). A water dispenser controlled by a solenoid valve, a buzzer speaker, a sensor hole and a light-emitting diode (LED) light were installed on the same side of the operant box and were controlled by a microcontroller system (Arduino Mega 2560; Arduino LLC, Italy). As a reward, a droplet of water was delivered through a pipe connected to the water dispenser. The tip of the pipe was placed 25 mm apart from the wall and 50 mm above from the floor. A droplet of water was being emitted for approximately

140 ms though the tip of the pipe and delivered to rats as reward. The volume of the reward was varied (0.04–0.05 ml) depending on the weight of each rat. The buzzer sounded for 1 s when the water emerged from the pipe so that the rats could immediately notice delivery of the reward regardless of their location or direction in the operant box. The rats' behaviors such as position and motion were monitored and recorded using a video camera (Microsoft Inc., USA). In addition, in order to precisely detect momentary changes in rat's bodily movements, a method of a three-axis accelerometer (MPU-6050; InvenSense Inc., USA) was used for three of the rats (see Robert et al., 2009 for details). The accelerometer was attached to the connector of the recording cable and the acceleration data of the rats' movements were acquired at 10 Hz of sampling rate in a range of ±4 gravitational force (g). Rats' bodily movements were recorded for 3 seconds prior to the reward deliveries and assessed as the signal vector magnitude (SVM) of the acceleration values (α) from each axis (x, y, z) (Fig. 2).

$$\mathrm{SVM} = \sqrt{{x_a}^2 + {y_a}^2 + {z_a}^2}$$

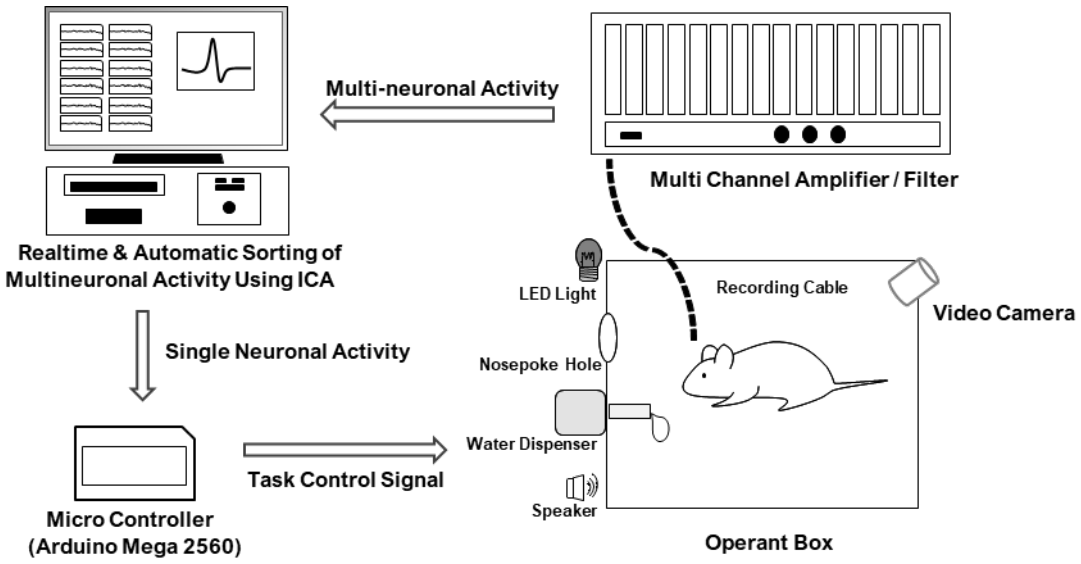

Fig. 2. Schematic diagram of the experimental system

The system mainly consists of a device for recording multi-neuronal activity, a PC for real-time spike sorting using independent component analysis (RASICA), and a microcontroller for task controls. The rat is connected to the system through a recording cable and can freely move in the operant box during training sessions.

2.3. Electrode design

The electrodes and microdrives were essentially identical to those used in the previous studies (Takahashi and Sakurai, 2005; 2007; 2009ab; Sakurai and Takahashi, 2013), except that each electrode bundle comprised of 6 or 12 tungsten microwires (12.5 μm diameter; California Fine Wire, CA, USA). Six or 12 tungsten microwires were bundled and inserted in a 33-gauge stainless-steel cannula (Small Parts, Miami, FL, USA). Each bundle was firmly fixed to the cannula so that the tip of the microwire bundle could penetrate brain tissue and be placed in the target cortical area without being bent. After fixation, the cannulas were again attached to the microdrive and implanted into a rat's motor cortex. The microdrive was designed to precisely adjust the depth of microwires using screws. Thus, the depth of the microwires in a cortex could be externally adjusted even after implantation. Normally, up to two microwire bundles were carried by a microdrive and the space between the bundles was kept within 500 - 700 μm in order to record signals from separate neurons in the same cortical area as possible. The impedance of the electrode was 200 - 500 kΩ. The tips of the microwires were cut with dedicated scissors leaving approximately 500 μm from the edge of the stainless-steel cannula just before surgery.

2.4. Pre-training (behavioral operant conditioning)

After the rats became accustomed to the experimenter sufficiently, simple behavioral operant conditioning, in which rats could learn the basic rules of operant conditioning, was used. At first, water was automatically delivered with buzzer sound at fixed intervals of 20 s. In this stage, associating the sound to water delivery, the rats became able to immediately recognize that water is accessible at their any positions and directions. The rats were then trained to poke their nose into the sensor hole (i.e., nose-poke response) to obtain the reward. The purpose of this stage was to allow the rats to learn that water could be obtained as a result of their voluntary actions. Finally, the LED light was turned on and off periodically at 5 mins intervals, and the reward was delivered only in

response to nose pokes when the LED light was on. Through this operant conditioning procedure, the rats learned that voluntary nose-poke responses only made during the “LED on” periods led to reward. The behavioral operant conditioning protocol was completed when the rats performed > 50 nose-poke responses in < 3 min during the LED-on condition and, for almost all rats, generally ended in a day. After behavioral training, surgery was performed for electrode implantation.

2.5. Surgery

After completion of behavioral operant conditioning, the electrode was surgically implanted into the rat’s primary motor cortex (+3.4 mm from bregma, 3.2 mm from mid-line) under anesthesia (isoflurane, approximately 2.5%). After the skull surface was exposed, 6 holes were drilled in the skull and anchor screws were installed into the holes. Dental cement was applied to the skull surface covering the anchor screws. After the cement was dried sufficiently, a hole for electrode insertion was made in the skull above the rat's primary motor cortex. The electrode then was fixed to the skull with dental cement at the point where the tips of the electrode were inserted into approximately 800 μm from the cortical surface. The hole was filled with Vaseline and sealed with dental cement. Rats were given a minimum of one week of recovery period.

2.6. Neuronal activity recording

2.6.1. Equipment

The neural activities recorded through the electrodes were firstly amplified through field-effect transistors (Toshiba, Tokyo, Japan) attached to the recording cable, then amplified and filtered by the multichannel main amplifiers (Nihon Koden, Kyoto, Japan). Finally, those activities were converted to digital signals via an analog to digital converter (National Instruments, Austin, Tx, USA) and finally processed and stored in a BMI PC (Dell, Tokyo, Japan).

2.6.2. Selecting target neuron

After recovery, multi-neuronal activity from the rats was recorded extracellularly, and spiking activity from individual neurons was detected using the custom-made software for real-time and automatic sorting of multi-neuronal activity using independent component analysis (RASICA) (Takahashi and Sakurai, 2005; 2013). The software operating RASICA was built using LabVIEW (National Instruments, Austin, Tx, USA) and MATLAB (Math Works, Tokyo, Japan). Stable long-term single-neuron activity for chronic neural operant conditioning was identified by lowering the tips of the electrodes up to approximately 90 μm - normally 30 μm per day. Three criteria were set for recruiting suitable neurons (target neurons) for conditioning: the amplitude and waveform of each spike from neurons were constant for at least 2 successive days; the mean firing rate was 3–8 Hz; and, finally, the signal-to-noise ratio (SNR) was > 3. For the above-mentioned second reason of criteria, the neurons with firing rates < 3 were excluded to avoid drastic changes in the performance by small amount of modification in firing rates. Likewise, the neurons with firing rates > 8 were not recruited to remove the possibility of overlapping problem in the identical window by their high firing rates. Only neurons that fulfilled all criteria were selected as targets for neuronal operant conditioning. Once a candidate for the target neuron was determined and no specific changes in those conditions were observed, the candidate neuron was finally set as the target neuron and neuronal operant conditioning task was operated.

2.6.3. Signal generation for task operation and processing by a microcontroller

Sampling rate for spike sorting by RASICA was 2500 and spikes were detected within a 10 ms and 5 ms overlapping window. Every time a spike was detected, the BMI PC transmitted a signal to the microcontroller in real time. The microcontroller then processed those signals and controlled the task based on the previously determined rules for neuronal operant conditioning.

2.7. Neuronal operant conditioning

2.7.1. Threshold setup

The number of spikes from each of the selected target neurons was counted as the rats moved freely, and the threshold was set for each target neuron for reward delivery. The recording time to obtain spike data to determine the thresholds was 20 s. As the identical rule, in which the threshold was set up based on spontaneous spiking activity within the fixed time span, was used to determine the threshold across all rats, every subject was required the equivalent level of enhancement in spiking activity to get reward. In order to compensate slight fluctuations in firing rates, 20 s of measurement was repeated for about 30 times. Also, the median instead of the mean was used as the threshold value since a drastic decrease in firing rates was observed occasionally when the rats were stationary (Fig. 3).

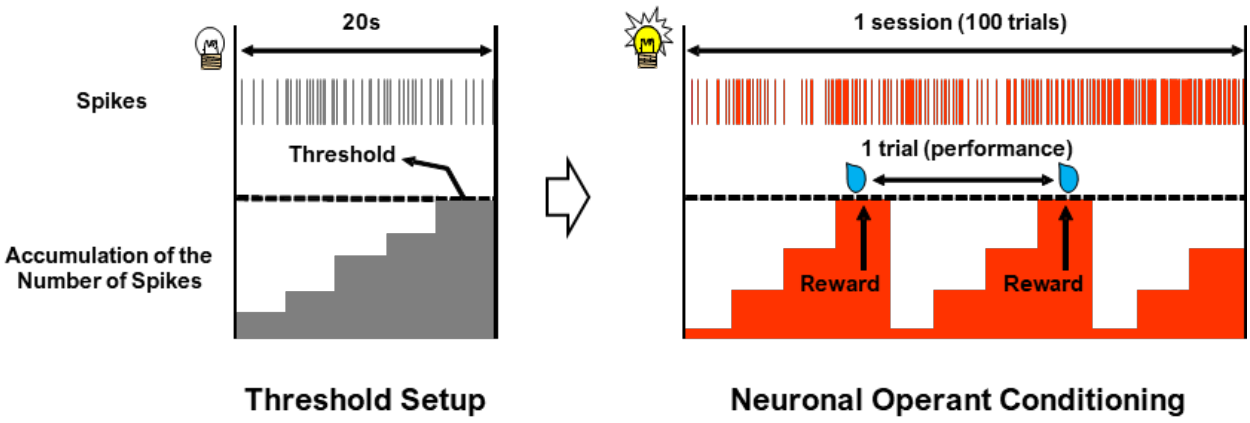

Fig. 3. Schematic diagram of the basic procedures for neuronal operant conditioning

Schematic diagram of the threshold set-up (left) and flows of neuronal operant conditioning (right) are shown. Prior to the first session of neuronal operant conditioning, the number of spikes from each target neuron was counted as the rats moved freely in the operant box, and the threshold for reward delivery was set for each rat. The length of one trial to obtain spike data to determine the thresholds was 20 s. In order to compensate fluctuations in firing rates, 20 s of measurement was repeated for about 30 times and the median was calculated as the value of a “reinforcement threshold”. During trials of neuronal operant conditioning, the rat was rewarded whenever the number of spikes counted from the target neuron reached the reinforcement threshold. In the FR schedule, the values of reinforcement threshold were fixed. In the VR schedule, on the contrary, the values of reinforcement threshold were randomly varied from trial to trial, ranging 60, 80, 100, 120, and 140% of the previously determined thresholds. A 1 s of ingestion period was imposed after reward delivery, and the spike counter was reset to zero. The next trial started without inter-trial intervals. One session of neuronal operant conditioning consisted of 100 trials (100 reward deliveries).

2.7.2. Schedule-based neuronal operant conditioning

In order to test the effect of different reinforcement schedules, the rats were divided into two groups: one group was trained with the FR schedule (n=3) and the other with the VR schedule (n=4). During a trial of neuronal operant conditioning, the rats were rewarded whenever the number of spikes from the target neurons reached a "reinforcement threshold". In the FR schedule, the values of reinforcement threshold were fixed and identical with previously determined thresholds, i.e., 100% of the threshold value was applied in all trials. In the VR schedule, on the other hand, the values of reinforcement threshold were not fixed, but randomly varied from trial to trial, ranging 60, 80, 100, 120, and 140% of the previously determined thresholds so that the rats could not predict the timing of reward deliveries. The values of reinforcement threshold in the VR schedule were pseudo-randomly distributed and quantitatively counterbalanced in every 20 trials and, therefore, the means of varied reinforcement thresholds in each session were always identical to those in the FR schedule (i.e., previously determined thresholds). Following reward delivery, a 1 s of ingestion period was imposed, and the spike counter was reset to zero and the next trial started without inter-trial intervals. One session consisted of 100 trials (100 reward deliveries) and rats were trained for two sessions per day. To maintain the motivation of the rats above a certain level, the second session in a day started after at least 8 hours later of the first one.

2.8. Histology and verification of electrode location

After all the experiments were completed, the rats were deeply anesthetized with pentobarbital injection (150 mg / kg) intraperitoneally. Then, 10% formalin solution was perfused to fix the brain tissue. The brain was removed and sliced at 50 μm intervals and the location of the electrodes were identified (Fig. 4).

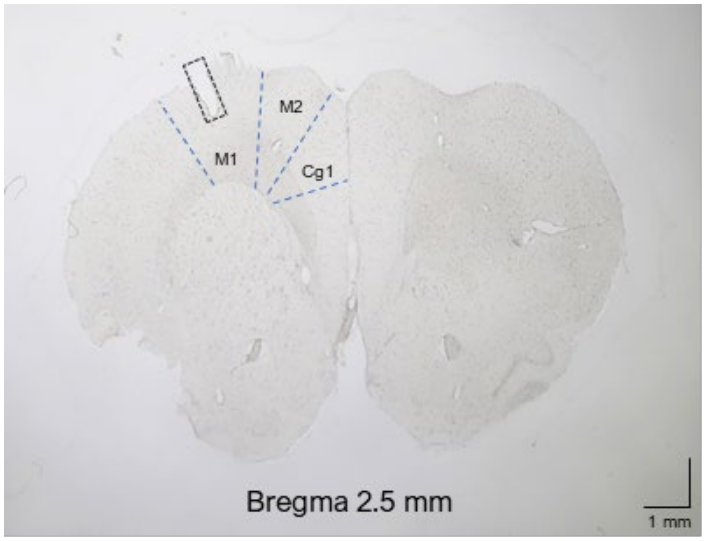

Fig. 4. An example of the electrode location in the brain tissue

The representative example of the brain tissue was shown. The brain was removed and sliced at 50 μm intervals after histology. The presented brain tissue was sliced at 2.5 mm from bregma. Trace of the electrode was found on the upper left corner of the photo and confirmed to be located in the primary motor cortex (M1).

Chapter 3. Results

3.1. General features of neuronal activity during operant conditioning

3.1.1. Chronic recording of neuronal activity

The neuronal operant conditioning procedures were conducted for 4 consecutive days, each of which had 2 sessions. During the whole sessions, 21 neurons from 9 rats were consistently recorded and used for data analysis (FR: n=5, VR: n=10, FR-VR: n=6). Each single neuronal activity was separated by spike sorting using Independent Component Analysis (ICA) from multi-neuronal activity recorded by the custom-made electrodes comprising 6 or 12 channels of microwires (Takahashi and Sakurai, 2005). Once the parameters for spike separation were calculated and stored in the BMI PC during the threshold setup, those parameters were identically applied to the multi-neuronal activity of each experimental session. During the neuronal operant conditioning conducted for 4 consecutive days, the relative peak amplitudes and the shape of waveforms were also visually checked, and no notable changes were observed. An example of separated four single neurons of the motor cortex that were successfully separated and identified from simultaneously recorded neural activity during operant conditioning is shown in Fig. 5 (see Takahashi and Sakurai, 2005; Sakurai and Takahashi, 2013 for detail). Nine of those separated neurons were set as target neurons (FR: n=3, VR: n=4, FR-VR: n=2) and the remaining 12 were set as non-target neurons and simultaneously recorded from the identical or neighboring electrodes recording the target neurons (FR: n=2, VR: n=6, FR-VR: n=4). The target neurons were selected based on the criteria described above among the neurons recorded simultaneously and were used for operant conditioning. As described in the Materials and Methods, only neurons with SNR > 3 were selected as the target neurons, and their shape of waveforms and relative peak amplitudes remained unchanged until the end of the last session on the 4th day. The other neurons with SNR > 2 were selected as non-target

neurons during the initial spike sorting process and those were not used for conditioning throughout the experiment. Neurons not satisfying those criteria were excluded from the recording.

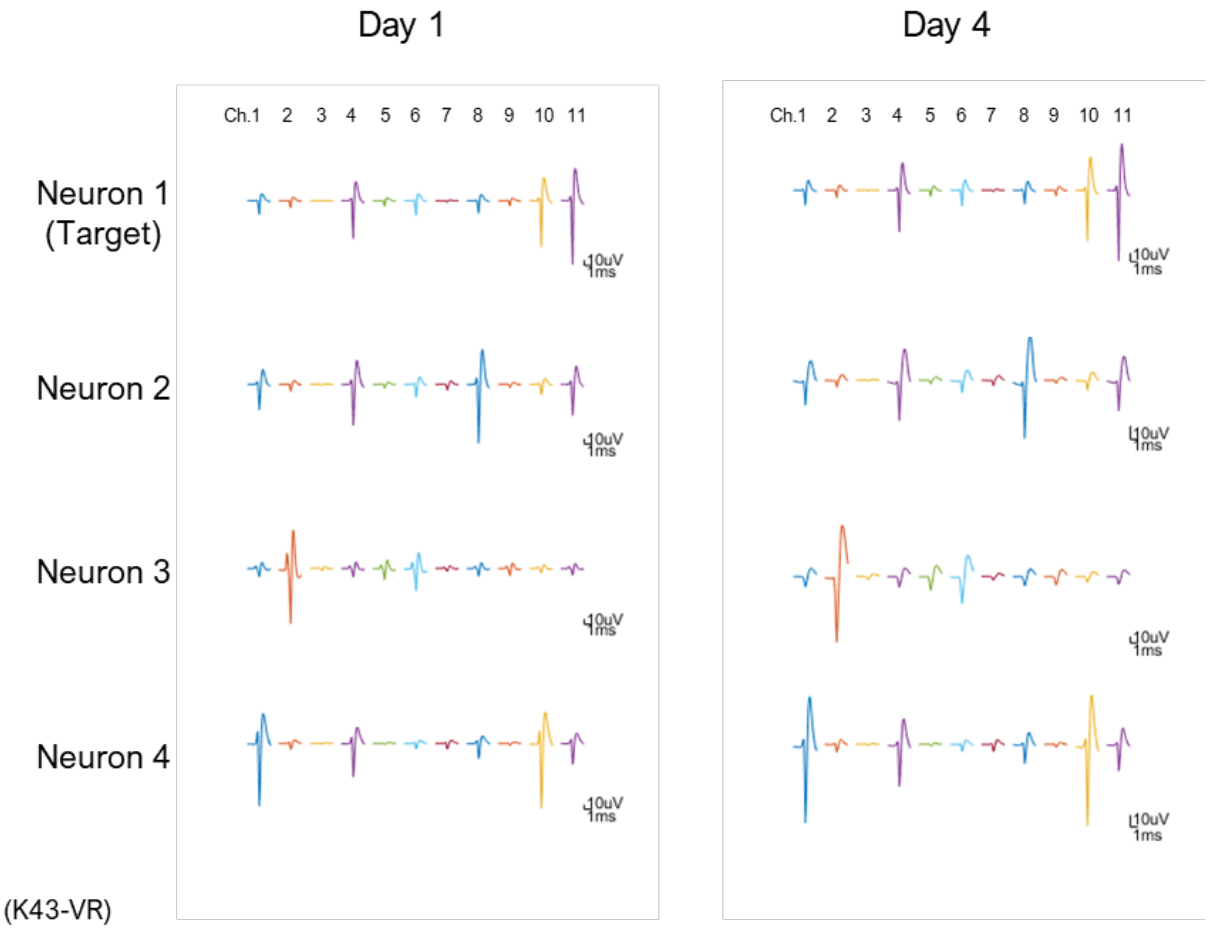

Fig. 5. An example of separated four single neurons of the motor cortex during operant conditioning

The averaged spike waveforms on each channel (microwires 1–11 in this example) of the electrode bundle during the first day (Day 1) and the last day (Day 4) of conditioning are plotted. Each row of 11 waveforms is determined to represent a single neuron by the spike-sorting system. The single neuron represented by the first row was the target neuron used for conditioning. All waveforms in each row including very small spikes also contributed to the calculation of spike-sorting. Throughout the 4 consecutive days of neuronal operant conditioning, no notable changes in the relative peak amplitudes and the shape of waveforms were observed.

3.1.2. Effect of specific rhythm on the activity of the target neuron

In order to verify the possibility that particular oscillatory rhythms of neurons could affect the enhancement of firing rates, the auto-correlations of all neurons used for analysis were calculated with 1 ms bins for a range of 128 ms. Subsequently fast Fourier transformation (FFT) was applied to the data to examine whether each neuron showed repeating firing pattern, i.e., rhythms at specific frequency or not. If a neuron had fired with a specific rhythm, the value of the power spectrum in FFT would show a peak around that frequency. As a result, we observed no specific rhythms in any neurons across all sessions (Fig. 6).

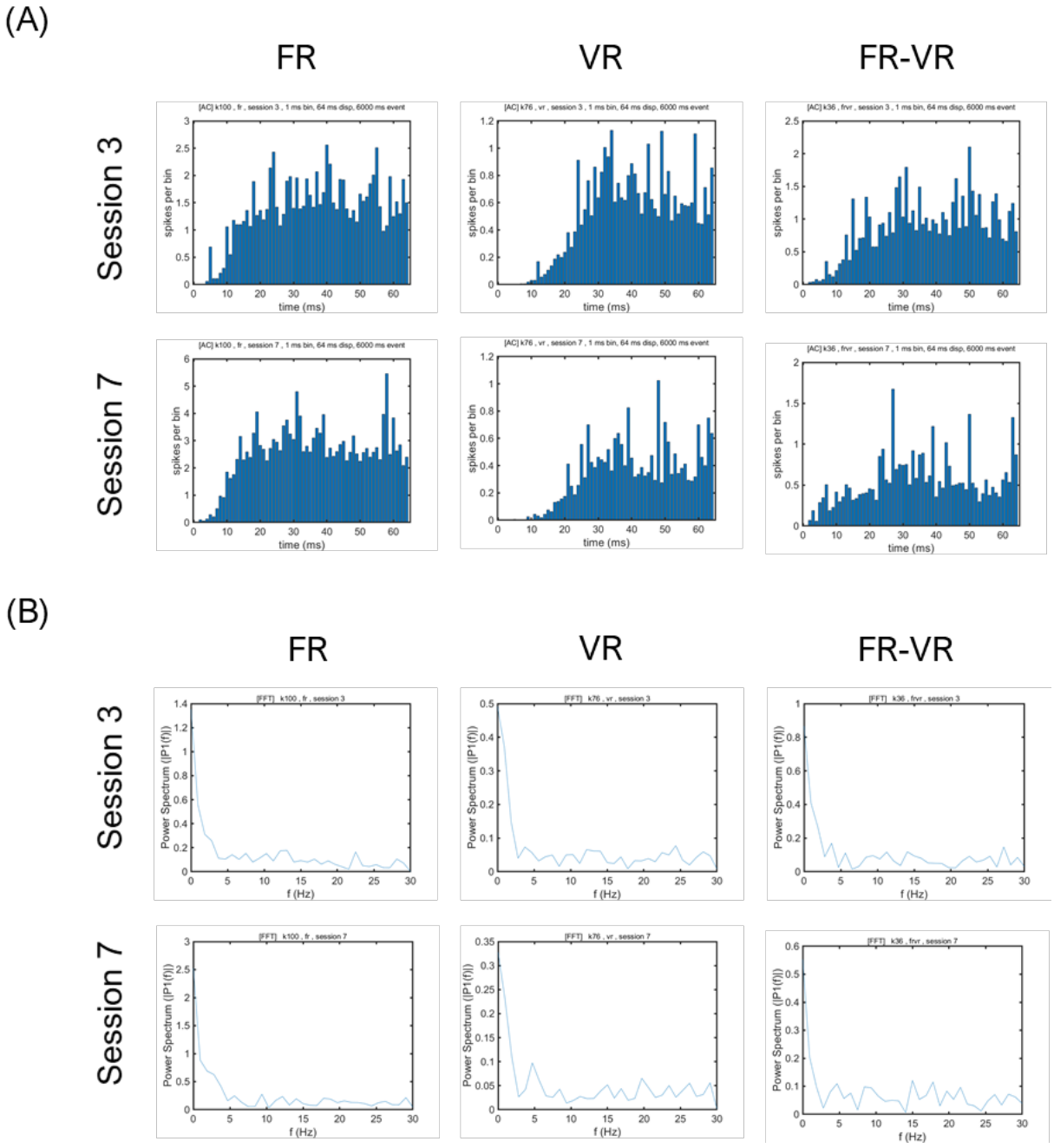

Fig. 6. An example of auto-correlograms and power spectrums of target neurons' spikes

(A) The auto-correlogram of the target neurons in session 3 and 7 for each schedule were shown. The vertical axis of each plot indicates the number of spikes per bin (1 ms). The horizontal axis indicates time (ms). (B) The power spectrums of the target neurons' spikes were plotted. Fast Fourier transform was applied on the results of auto-correlogram in (A) to examine if a specific rhythm of spiking affected the enhancement of the firing rates in target neurons. The vertical axis of each plot indicates the power spectrum and the horizontal axis indicates frequency (Hz). No specific frequency was observed during neuronal operant conditioning.

3.1.3. Movement verification

Among all data, a review of the video recordings of the rats' bodily movements provided no clear evidence of specific behavioral changes during neuronal operant conditioning. We analyzed the data of fine bodily movements detected by the three-axis accelerometer from three of the rats using simple linear regression analysis for finding entire changes in momentum and ANOVA for transient movement. The result showed no clear behavioral changes around reward delivery during the neuronal operant conditioning (Fig. 7).

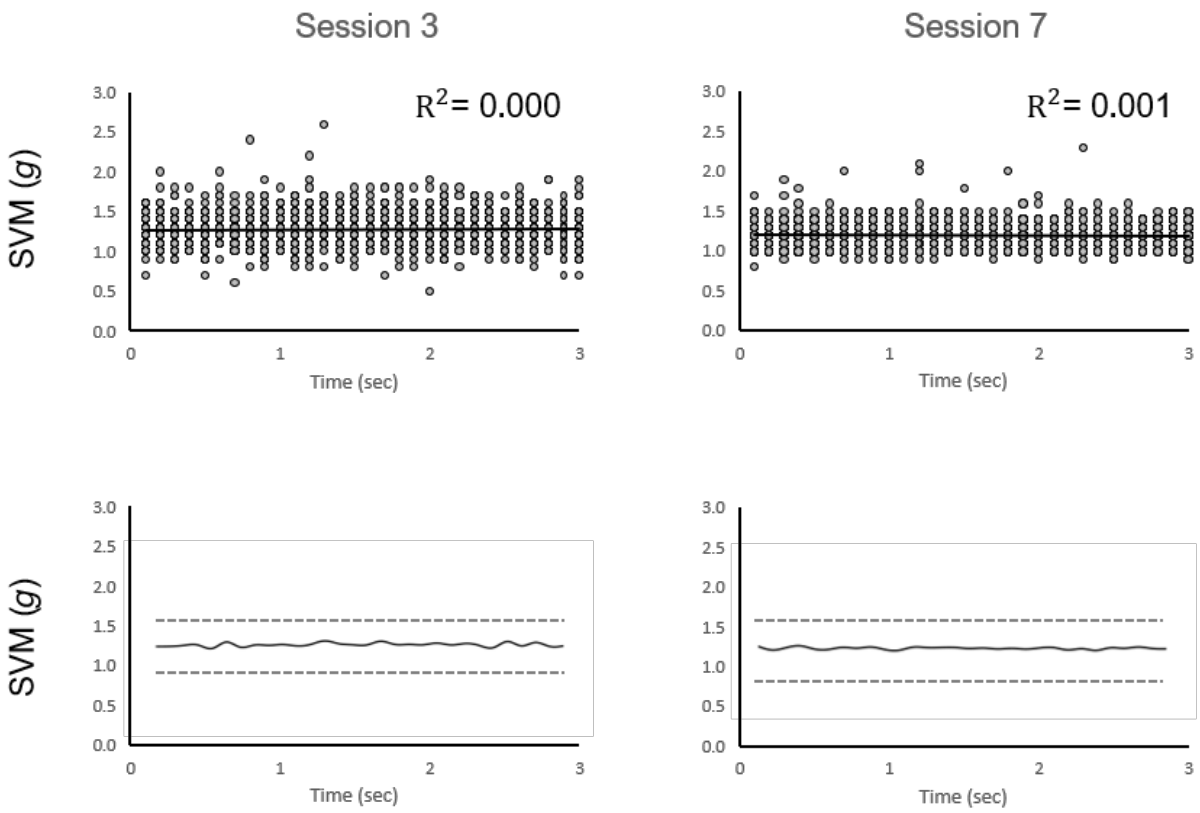

Fig. 7. An example of bodily movements detected by the three-axis accelerometer

The three-axis accelerometer recorded the fine movements of the rats. The data from one of the rats during the second day (Session 3, left) and the last day (Session 7, right) of the neuronal operant conditioning using FR schedule was analyzed and plotted. The two top plots are the result of simple linear regression analysis and the two bottom plots indicate the mean of each signal vector magnitude (SVM) value at the bin and 95% of confidence interval. The vertical axis of each plot indicates SVM of 3 axes (x, y, z) in gravitational force (Robert et al., 2009). The horizontal axis indicates time in second just prior to reward delivery. The dots in top plots represent data of all trials in each session (100 trials) and thus one dot sometimes includes data of several trials. The horizontal straight lines represent results of linear regression analysis to check specific changes in the level of movements of the rat. Session 3 in the second day was used for the data in the early period of conditioning, because the rats were not fully accustomed to the experimental procedure in the first day and had a possibility to show irregular and unusual movements.

3.2. Neuronal operant conditioning – Behavioral performance-based analysis

3.2.1. Procedures of analysis

The time intervals between reward deliveries—not including the 1 s of ingestion period—were used as the values of "performance" of the neuronal operant conditioning. Only the performance values obtained at 100% of threshold were used for comparison between the FR and VR schedules, i.e., the values of performance recorded in all trials were used in the FR schedule and those in only 20 trials using 100% of threshold were used in VR schedule for comparison. This procedure was employed to directly compare the effects of the FR and VR schedules on performance, although the average of the reinforcement thresholds in all trials in the VR schedule was 100%.

3.2.2. Effects of different schedules on the performance and interactions between time and schedule

To test how the different reinforcement schedules affected neuronal operant conditioning in both groups generally, we analyzed the performance of the rats using two-way repeated measures ANOVA (Fig. 8). In order to reduce possible influences such as time when the tasks were conducted or changes in rat's motivation, the data of two sessions per day were used for analysis. The ANOVA demonstrated only marginal significance in the main effect of reinforcement schedules ($F(1,5) = 4.98$, $p = 0.076$) as well as in interaction between reinforcement schedules and conditioning days ($F(1.94,9.72) = 3.17$, $p=0.088$) in the total period of conditioning. However, the Bonferroni post-hoc analysis indicated that the performance in the FR schedule was significantly higher compared with the VR schedule ($p<0.05$) on the last day of conditioning, suggesting that the difference in the performance between both schedules could not have been induced without the effect of different schedules.

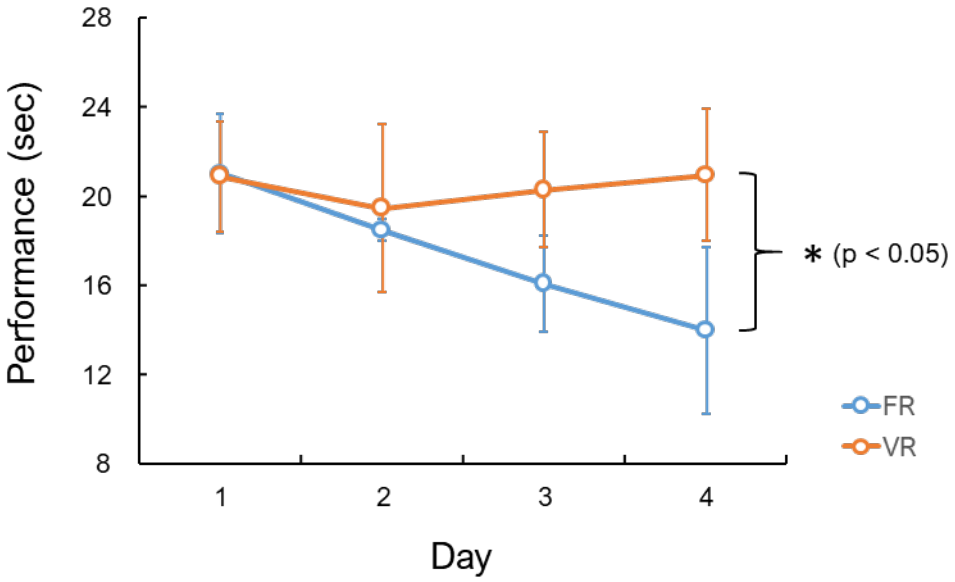

Fig. 8. The interaction of different reinforcement schedules and time

The interaction of different reinforcement schedules (FR and VR) and time (day) on the rats' performance across four successive training days was tested with repeated measure of two-way ANOVA and plotted. The rats were trained for two sessions per day, and the data from two sessions in one day were used for statistical analysis. The vertical axes indicate performance, interval times taken for the rats to obtain a reward in each trial and, therefore, the lower value means the better performance.

3.2.3. Performance comparison in each schedule

We could observe the effect of different schedules on the performance shown in 3.2.2. To make sure that the difference was related to the enhancement of the performance in the FR schedule group, we compared the performance in each schedule respectively. The mean time intervals between reward deliveries in the FR schedule decreased from 21.01 s on the first day to 13.98 s on the last day of conditioning (33.46% decrement), meaning there was an enhancement in the performance. In VR schedule, the time intervals showed almost no increment (from 20.87 to 20.94 s) during the same period, indicating that there was little change in the performance. A t-test was conducted to confirm the different results of the schedules on performance (Fig. 9). Compared to the first day, a statistically significant enhancement in the performance was observed on the final day of operant conditioning in the FR schedule ($t(4) = 3.08$, $p<0.05$). However, there was no significant difference between the performances on the first and the last days in the VR schedule ($t(6) = -0.03$, n.s.). These results suggest that during the operant conditioning, the rats in the FR schedule learned to modulate their neurons' firing to obtain rewards. On the contrary, the rats reinforced by the VR schedule exhibited no such learning until the last day of the conditioning. The comparison of the effects of the different reinforcement schedules revealed that the performance of each neuron was differentially affected by each schedule.

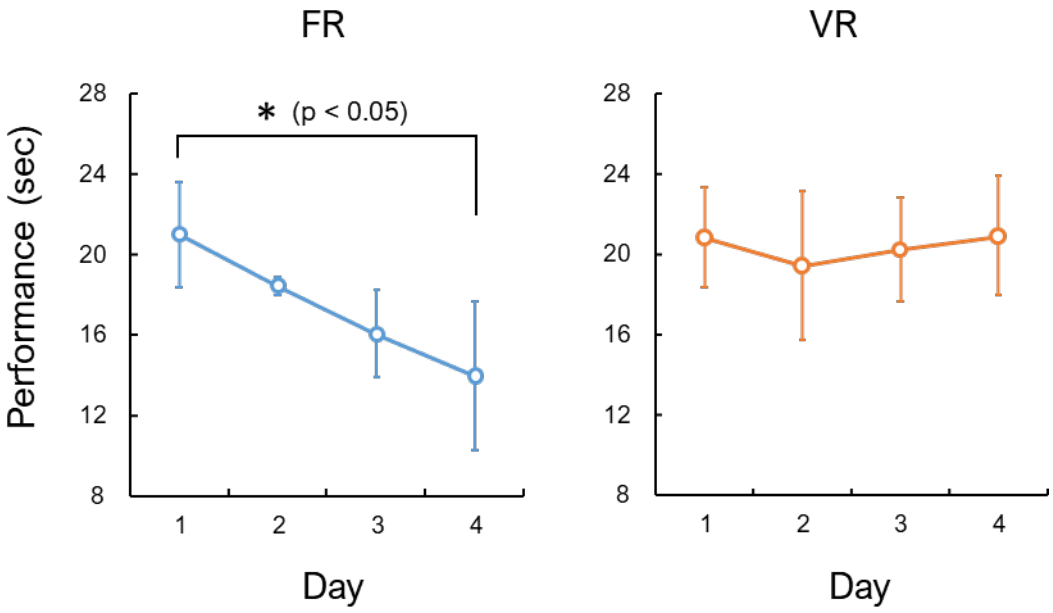

Fig. 9. The effect of different reinforcement schedules on each rat's performance

The performance of rats in the fixed ratio (FR, left) and variable ratio (VR, right) schedules during neuronal operant conditioning was shown. Details such as training session, day and the value of performance are basically identical with Fig. 8. Contrary to Fig. 8, however, a t-test was used to examine the effect of the different schedules on the subjects in each group respectively. The values of the performance on the last day of conditioning was tested against the ones on the last day in each group respectively.

3.2.4. Successive FR-VR schedule as an additional experiment

Neuronal operant conditioning with successive FR-VR schedules on the same neurons was also performed to test the effect of different reinforcement schedules and find further possible explanation for the poor performance in the VR schedule. The FR schedule was applied during the first two days and the VR schedule during the last two days (n=2). Due to insufficient subjects, we examined a potential factor which might have affected the performance of the neuronal operant conditioning by calculating the slope of the performances. Simple linear regression analysis was used for the comparison of the performance during four consecutive days (Fig. 10). The analysis of the FR, VR, and FR-VR data demonstrated that the recorded neurons in the FR-VR schedules improved their performance ($y = -2.6835x + 25.168$, $R^2 = 0.645$, $p<0.05$), which was similar in FR schedule ($y = -2.3468x + 23.243$, $R^2 = 0.68$, $p<0.001$). This result was noticeable compared with the poor performance in the VR schedule alone ($y = 0.1022x + 20.126$, $R^2 = 0.002$, n.s.), implying that the VR schedule may lead to successful operant conditioning of neuronal activity by being combined with different reinforcement schedules such as the FR schedule. Since nothing can be clearly concluded at this stage without performing statistical tests with enough data, it needs to be tested in the future research.

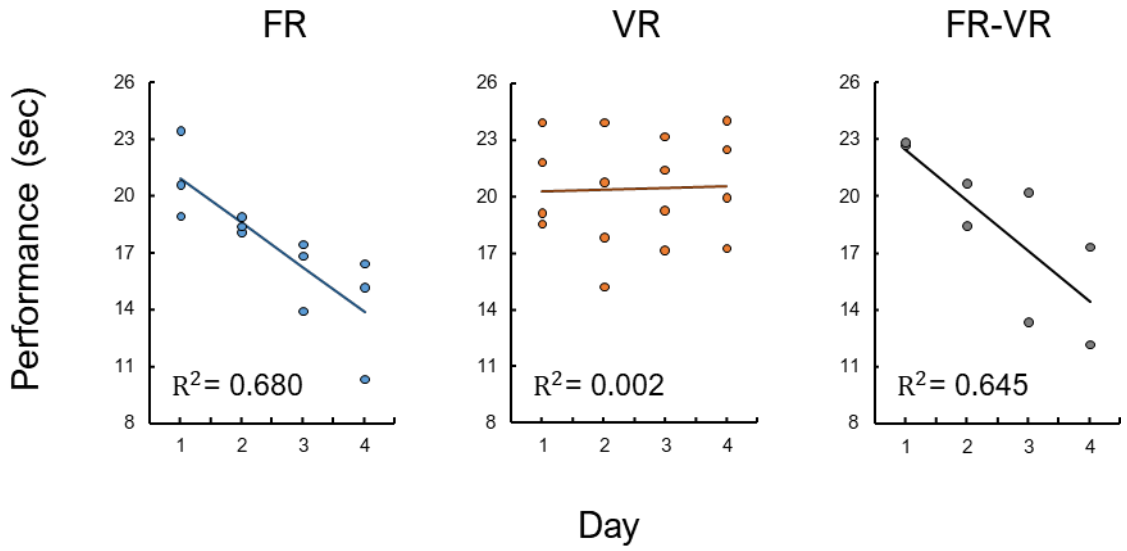

Fig. 10. The effect of the successive FR-VR schedule

To examine the effect of different schedules on the same neuron, a successive FR-VR reinforcement schedule was applied to two rats. Except the reinforcement schedule, other details were identical with FR and VR schedules (See Fig. 8 and 9). Linear regression analysis for FR alone (left), VR alone (middle), and successive FR-VR (right) schedules was used and each performance was plotted.

3.2.5. Analysis using the entire reinforcement thresholds in the VR schedule

In the present study, the comparison of behavioral performance during neuronal operant conditioning between the FR and the VR schedules was conducted using the data obtained at 100% of reinforcement threshold in both schedules. However, the unused data might have affected the results. Therefore, we conducted the identical analysis (see Result. 3.2.2 and 3.2.3) on the data using the entire reinforcement thresholds in the VR schedule. The ANOVA demonstrated the main effect of reinforcement schedules ($F(1,5) = 7.59$, $p < 0.05$) as well as the interaction between reinforcement schedules and conditioning days ($F(2.49,12.49) = 4.59$, $p < 0.05$) in both schedules during the total period of conditioning. Also, the Bonferroni post-hoc analysis indicated that the performance in the FR schedule was significantly higher compared with the VR schedule ($p<0.05$) on the last day of conditioning, as well as the third day of conditioning ($p < 0.05$), suggesting that the different schedules affected more critically on the performance of the entire trials rather than that of the trials at 100% reinforcement threshold only. Although the result of the latter analysis was not in opposition to the former one, this issue needs to be investigated in detail in the future research with sufficient subject and various analysis.

3.3. Analysis of individual neurons' activity

3.3.1. General features and the purpose of analysis

In behavioral operant conditioning, it is well known that different types of reinforcement schedules cause different patterns of response rate after reward delivery, e.g., scallops of response rates in FR schedule (Reynolds, 1975). To test if those kinds of different patterns exist in the neuronal activity, firings of the individual target and non-target neurons during the neuronal operant conditioning were analyzed off-line in addition to the analysis of the performance described above. We examined in detail how the individual neurons acted during a session using several components comprising firing pattern such as mean firing rates and time to peak firing rates from trial onset. As a result, no significant changes in neuronal activity between session 1 and session 7 were found using a paired t-test except for mean firing rates and time to peak firing rates in the FR schedule (Table 1).

Schedule	Tested points	Session 1	Session 7	Significance
FR	Mean firing rates (Hz)	8.85	15.28	P < 0.05
	Peak firing rates per bin (100 ms)	0.98	1.94	P < 0.05
	Min firing rates per bin (100 ms)	0.75	1.19	n.s.
	Time to peak firing rates from trial onset (ms)	3666	1733	n.s.
	Time to valley firing rates from trial onset (ms)	1467	1433	n.s.
	Peak – Min firing rates (z-score)	4.25	4.34	n.s.
VR	Mean firing rates (Hz)	9.83	9.69	n.s.
	Peak firing rates per bin (100 ms)	1.17	1.15	n.s.
	Min firing rates per bin (100 ms)	0.76	0.63	n.s.
	Time to peak firing rates from trial onset (ms)	1525	2600	n.s.
	Time to valley firing rates from trial onset (ms)	1150	2050	n.s.
	Peak – Min firing rates (z-score)	4.20	4.16	n.s.

Table 1. Examination of the activities of individual neurons

The values such as mean firing rates between session 1 and session 7 in FR and VR schedules were examined using paired t-test. The values of the target neurons were averaged and presented in the table. No significant changes between session 1 and session 7 were observed except for mean firing rates and time to peak firing rates from trial onset in the FR schedule.

3.3.2. Comparison of mean firing rates between the FR and VR schedules

The neuronal operant conditioning was operated based on the signals generated by RASICA that detected and sorted spikes from the recorded neuronal activity, and the analysis of behavioral performance was mainly related to the entire changes of firings in a relatively long time span (a trial). Therefore, the mean firing rates of individual neurons in controlled period were needed to be analyzed. The activities of target neurons 3 s before and after reward delivery, the time span that never overlaps with neighboring trials and evenly includes the neuronal activity in each phase (during trial and after reward), were plotted and analyzed to verify how the activity of individual neurons was changed and reflected to the performance of the rats during the neuronal operant conditioning with the FR and VR schedules (Fig. 11 and 12). We firstly used the mean firing rates for three seconds before and after reward delivery for the comparison (Fig. 13). In the FR schedule, the mean firing rates increased from 9.1 Hz in session 1 to 15.44 Hz in session 7. The increment was statistically significant (paired t-test, $t(2) = -10$, $p<0.05$). However, no significant change in mean firing rates was observed between session 1 and 7 in the VR schedule (paired t-test, $t(3) = -0.44$, n.s.).

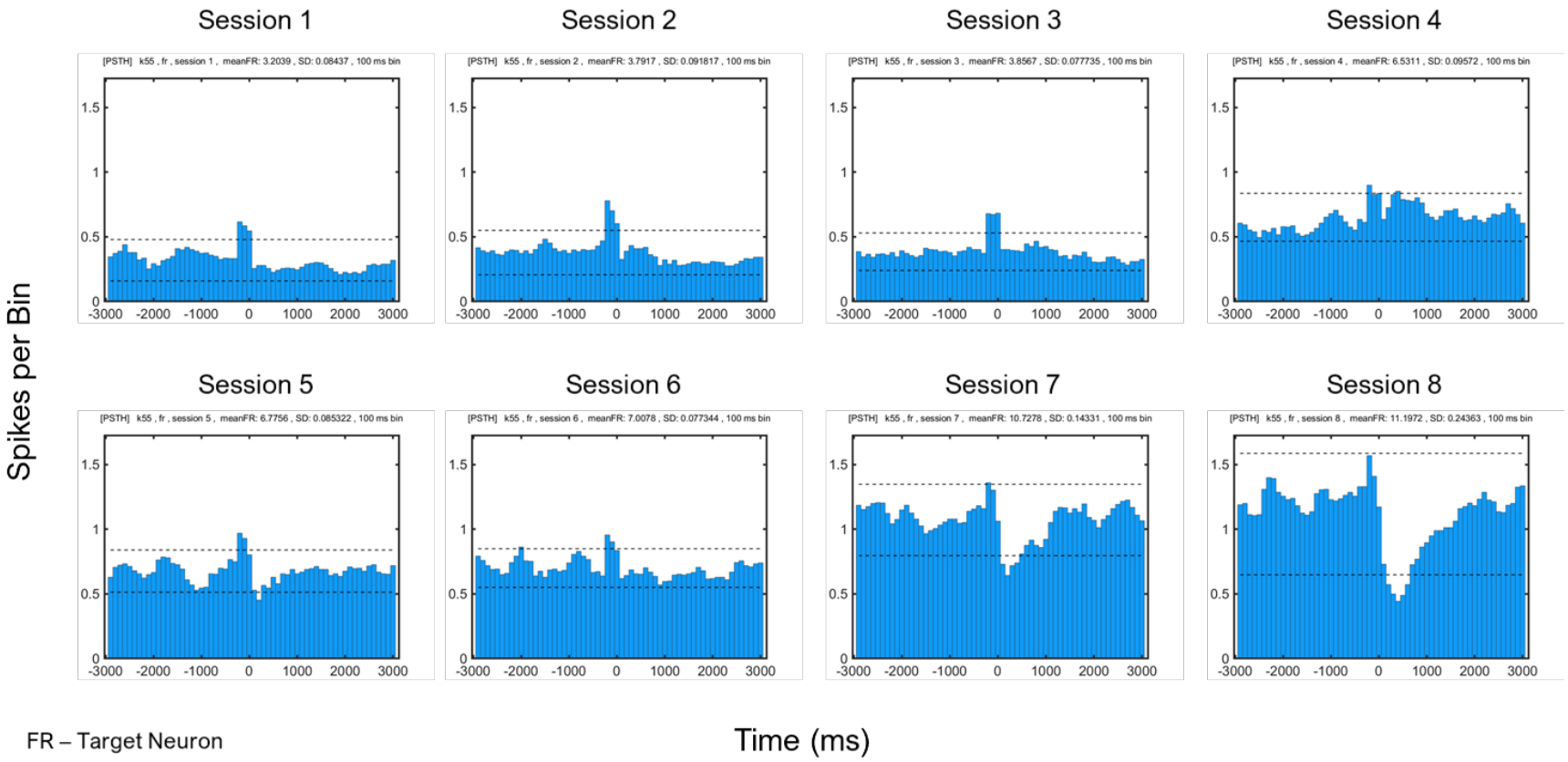

Fig. 11. An example of the target neuron's activity in the FR schedule

Peri-stimulus time histograms (PSTHs) of the target neuron's activity in the FR schedule around reward delivery across four successive training sessions (k55) were shown. The vertical axes indicate the number of spikes per bin (100 ms). The horizontal axes indicate the time (ms). Displayed range of each histogram is 3 s before and after reward delivery. 95% of confidence intervals were also calculated after 1000 times of bootstrap process and plotted.

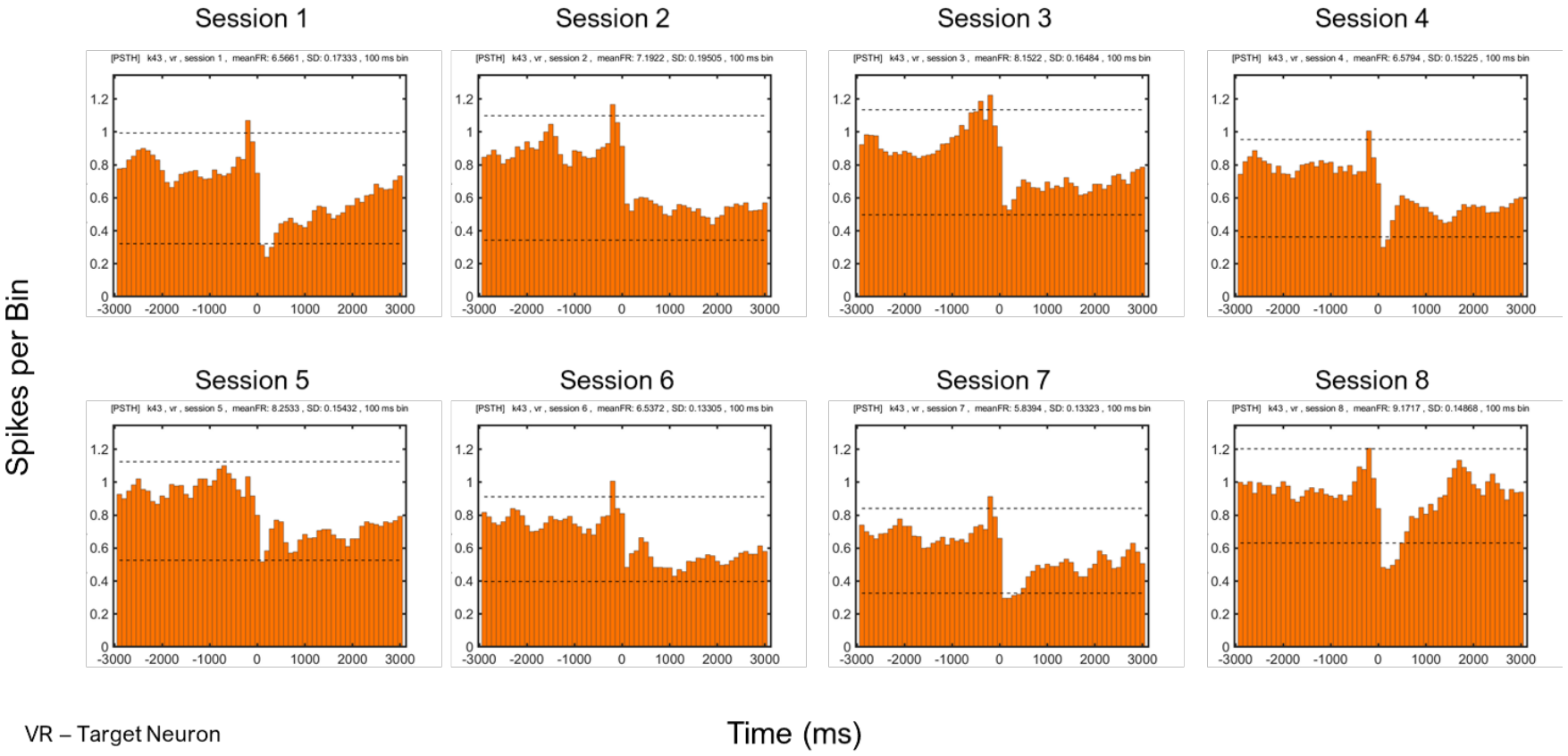

Fig. 12. An example of the target neuron's activity in the VR schedule

PSTHs of the target neuron's activity in the VR schedule around reward delivery across four successive training sessions (k43) were shown. Except the plotted neuron, every detail is identical with Fig. 11.

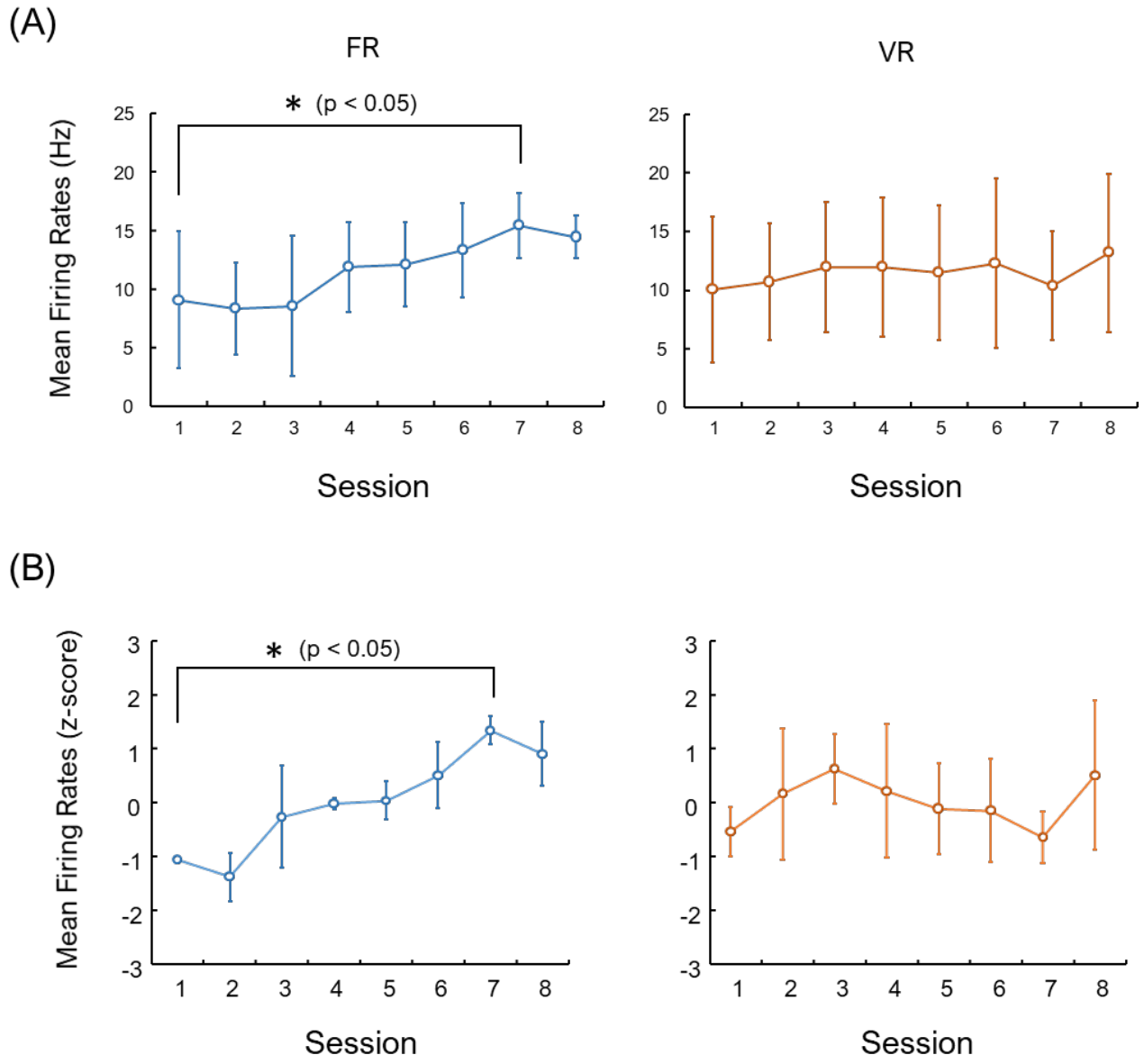

Fig. 13. Comparison of mean firing rates of the target neurons in the FR and VR schedules

(A) To examine if the change of raw neuronal activity was sufficiently reflected to the performance using RASICA, comparison of the mean firing rates between the first and the last day of conditioning (session 1 and 7) was conducted in each schedule. The vertical axes indicate mean firing rates (Hz) of the target neuron during neuronal operant conditioning. (B) Mean firing rates used in (A) were normalized and plotted. The vertical axes indicate mean firing rates in z-score.

3.3.3. Comparison of peak firing rates between the FR and VR schedules

Subsequently, in order to identify any specific patterns in neuronal activity after reward delivery, peak firing rates of individual neurons during 6 seconds after reward delivery in the FR and VR schedules were calculated (Fig. 14). The peak firing rate of neurons after reward delivery in session 7 increased significantly compared to session 1 only in the FR schedule condition (paired t-test, $t(2) = -11.89$, $p<0.01$). We also plotted peri-stimulus time histograms (PSTHs) of the activity of each neuron for 6 seconds after reward delivery. The sessions used for the calculation were identical to those for the firing-rate histograms. Each bin was 100 ms and the y axis indicates the number of spikes per trial. Besides the peak firing rate, various firing patterns of target neurons were observed around reward delivery. These patterns were decreasing, increasing or being newly created or vanished as the operant conditioning progressed. Although some statistical analysis may be able to systematically quantify the various aspects of firing patterns such as temporal latency or valley shapes after reward delivery, we could not observe at present any constant schedule-dependent firing patterns of the neurons except the mean firing rates and the peak firing rates in the FR schedule.

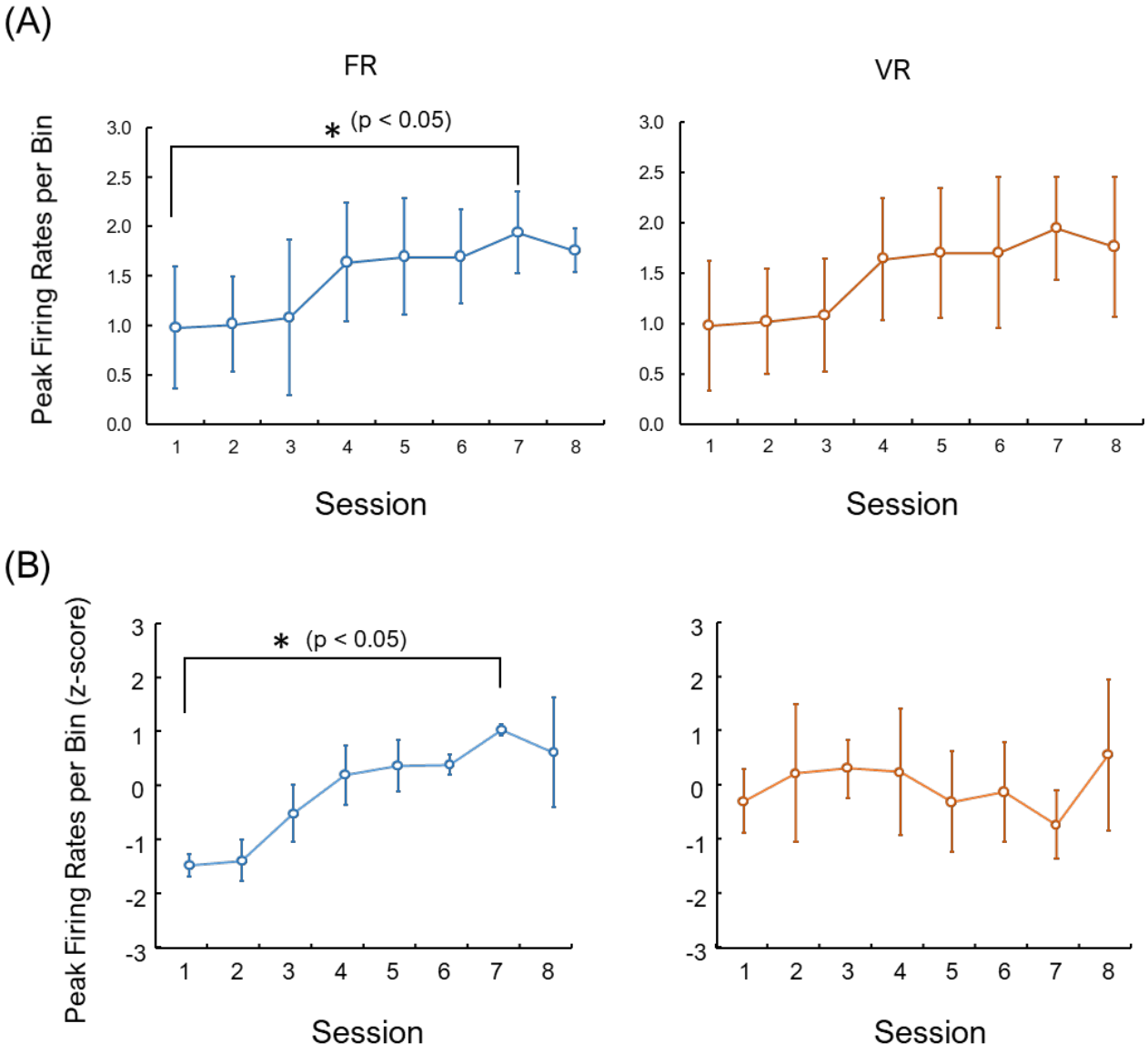

Fig. 14. Comparison of peak firing rates of the target neurons in the FR and VR schedules

(A) To examine if any specific patterns in neuronal response existed during the conditioning, the comparison of peak firing rates per bin (100 ms) between the first and the last day of conditioning (session 1 and 7) was conducted in each schedule. The vertical axes indicate peak firing rates per bin (the number of spikes per 100 ms bin in a trial) of the target neuron during neuronal operant conditioning. On the contrary to mean firing rates, peak firing rates is not directly related to the performance of the rats. (B) Peak firing rates per bin used in (A) were normalized and plotted. The vertical axes indicate peak firing rates per bin in z-score.

3.3.4. Activity of non-target neurons

Though the activity of the target neurons could be estimated through the performance, one of the non-target neurons was not observed even in indirect form. Therefore, we also verified the activity of non-target neurons recorded simultaneously with the target neurons. There was no statistically significant enhancement in firing rate for the non-target neurons, which was not related to the performance of conditioning (Fig. 15). This result was consistent even in FR schedule, in which the target neurons were successfully conditioned, indicating that it was possible for the target neurons to be selectively conditioned and changes in their firing rates were not directly correlated to firing rates in their neighboring neurons. Regarding spike patterns, various features in the firing pattern were also observed before and after reward delivery during the neuronal operant conditioning for the non-target neurons. Likewise, we could not identify constant tendency in the various firing patterns (Fig. 17 and 18), even in the peak firing rates in the FR schedule (Fig. 16).

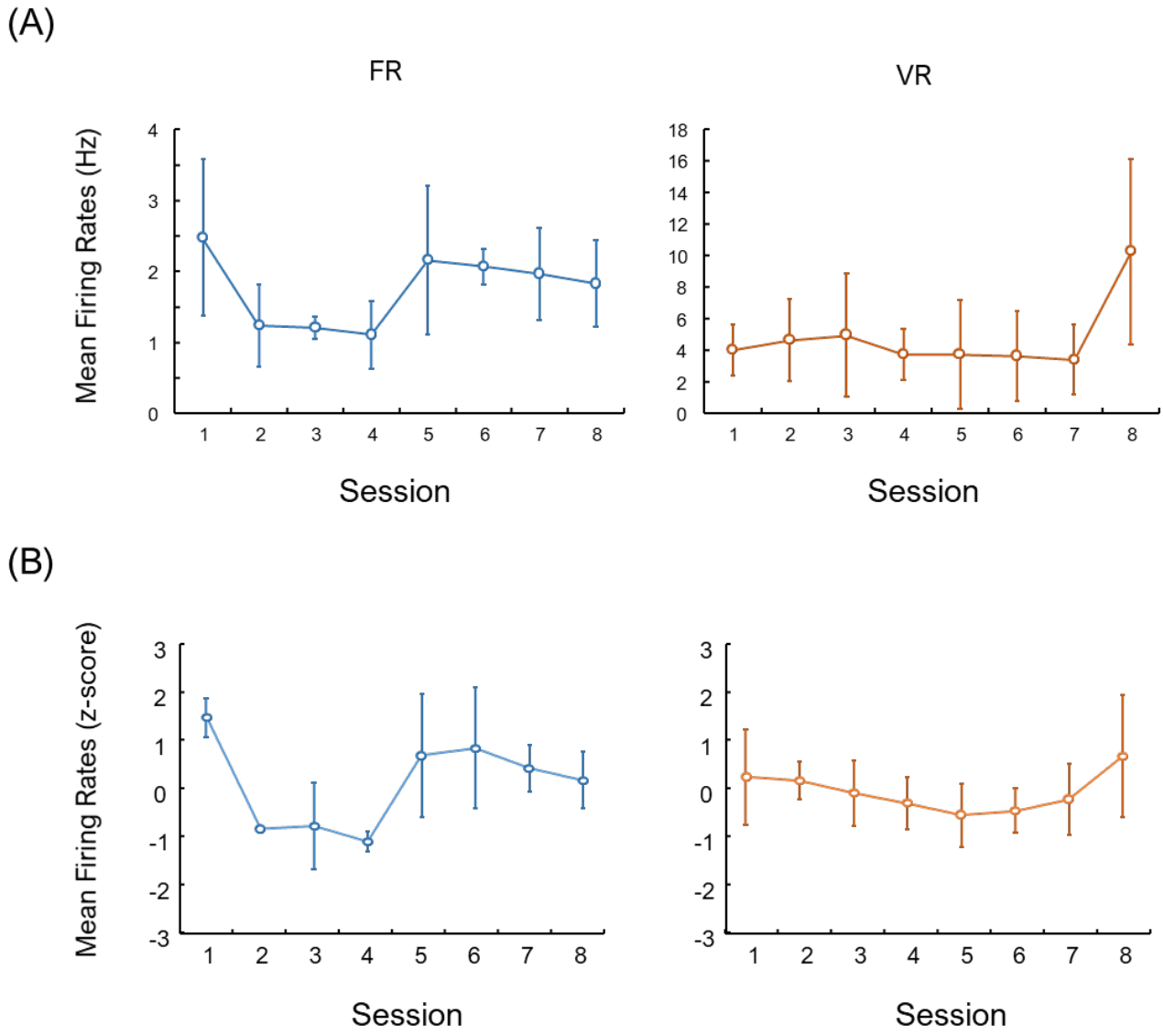

Fig. 15. Comparison of mean firing rates of the non-target neurons in the FR and VR schedules

(A) To examine if the activity of non-target neurons had been changed in relation to the one of target neurons, the performance comparison between the first and last day of conditioning (session 1 and 7) using mean firing rates was conducted in each schedule respectively. The vertical axes indicate mean firing rates (Hz) of the non-target neuron during neuronal operant conditioning, therefore, the higher value means the better performance. (B) Mean firing rates used in (A) were normalized and plotted. The vertical axes indicate mean firing rates in z-score.

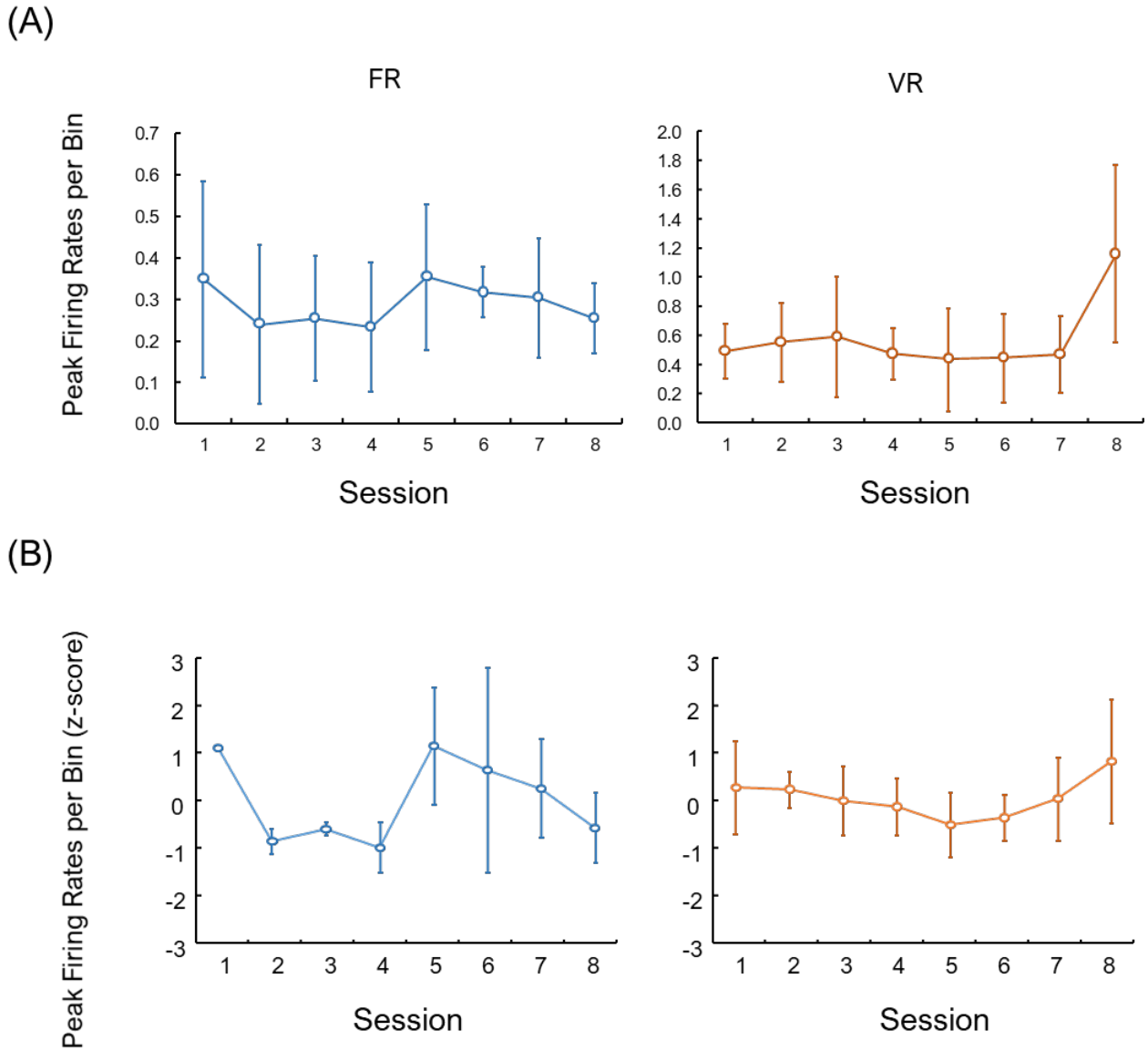

Fig. 16. Comparison of peak firing rates of the non-target neurons in the FR and VR schedules

(A) To examine if any specific patterns in neuronal response existed during the conditioning, the performance comparison between the first and last day of conditioning (session 1 and 7) using peak firing rates per bin (100 ms) was conducted in each schedule respectively. Except the subject of the test, every detail is identical with fig. 14. (B) Peak firing rates per bin used in (A) were normalized and plotted. The vertical axes indicate peak firing rates per bin in z-score.

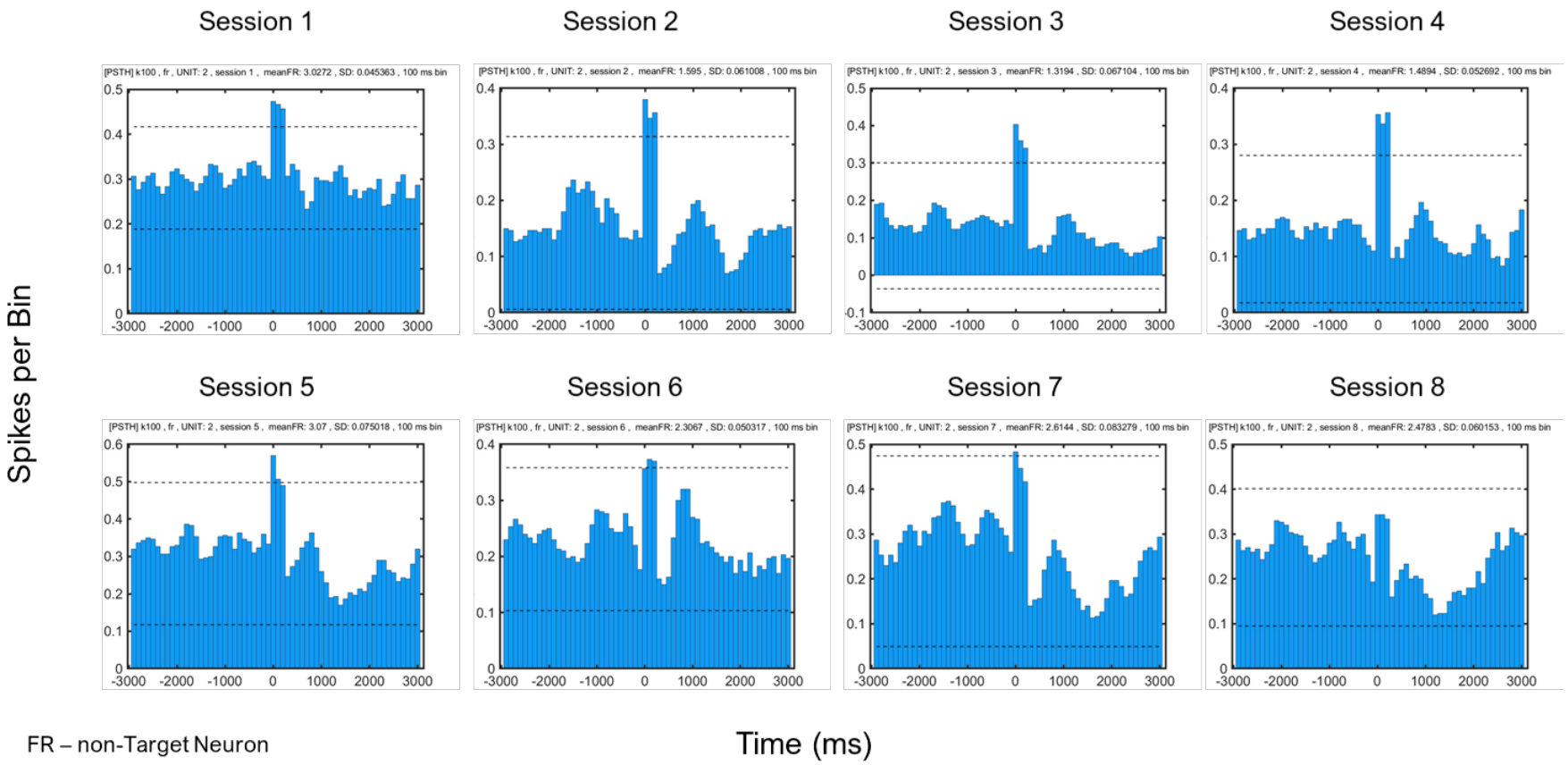

Fig. 17. An example of the non-target neuron's activity in the FR schedule

PSTHs of non-target neuron's activity in the FR schedule around reward delivery across four successive training sessions (k100) were shown. Except the plotted neuron, every detail is identical with Fig. 11.

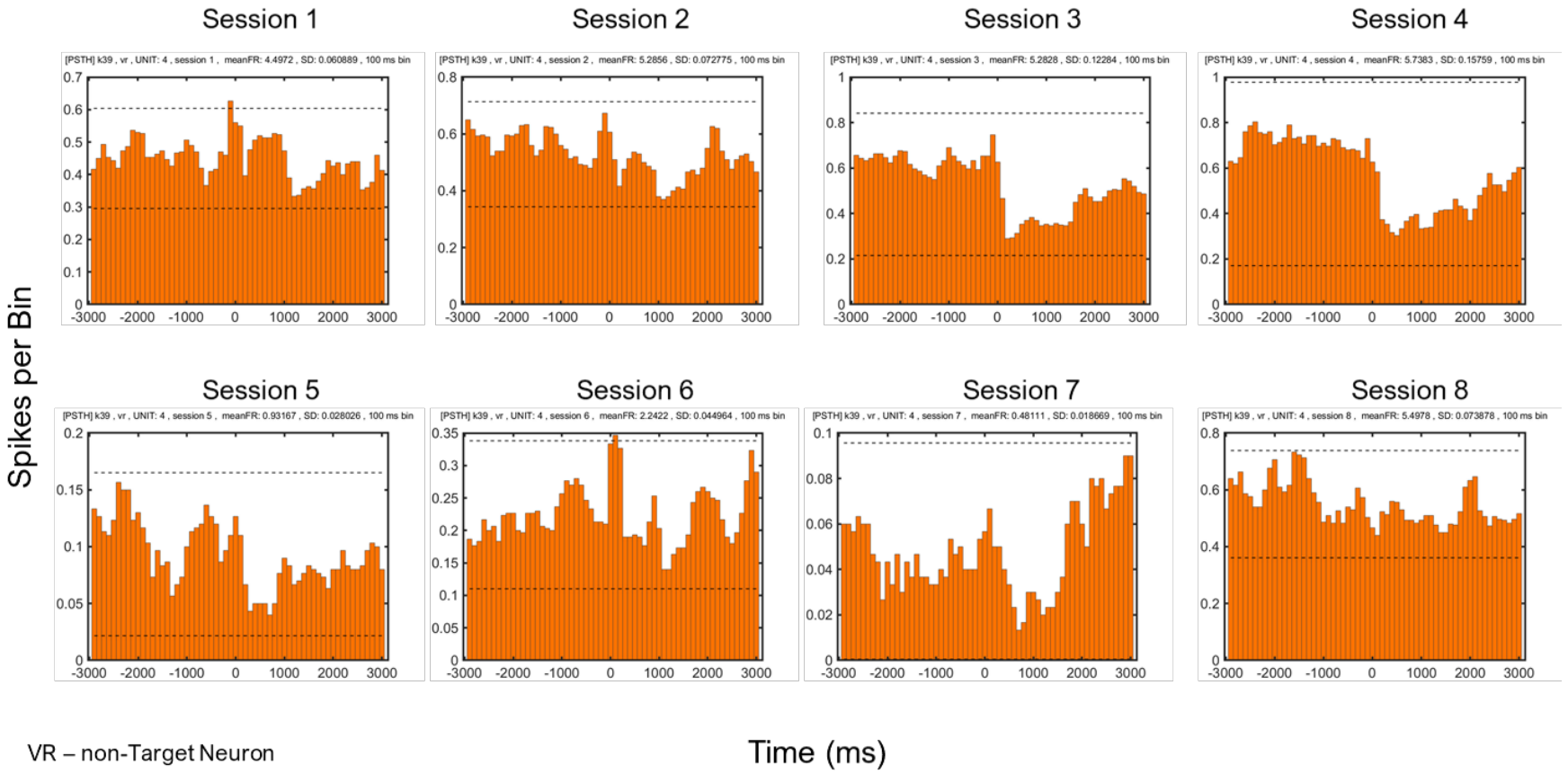

Fig. 18. An example of the non-target neuron's activity in the VR schedule

PSTHs of non-target neuron's activity in the VR schedule around reward delivery across four successive training sessions (k39) were shown. Except the plotted neuron, every detail is identical with Fig. 11.

Chapter 4. Discussion

4.1. Neuronal operant conditioning in the previous and the present studies

Most previous studies investigating neuronal operant conditioning used a continuous reinforcement schedule (CRF) or simple intermittent reinforcement schedules in discrete type situations (Arduin et al., 2013; Engelhard et al., 2013; Sakurai and Takahashi, 2013). This study was the first to investigate the effect of basic and different reinforcement schedules in the free operant type situation on neuronal operant conditioning using long-term recording of single neuronal spiking for four days of training. The result demonstrated that neuronal activity was successfully conditioned in the FR schedule but not in the VR schedule when they were applied individually. This result was unexpected because the responses of animals can be easily conditioned in both schedules, and the conditioned response rates are generally higher in VR than in FR schedule in behavioral operant conditioning (Reynolds, 1975). However, we could observe the rats in the FR-VR schedule showed very similar tendency in the result to the one in the FR schedule using linear regression analysis. Although further experiments with sufficient subject need be done to clarify the observation, this comparison also implies that the training in the FR schedule could possibly “shape” rat’s responses applicable in the VR schedule.

4.2. Activity of the individual neurons

We performed neuronal operant conditioning on single neurons in the primary motor cortex of the rats. Among the recorded neurons, we observed various firing patterns around reward delivery. Some neurons showed bursting activation within about 300 ms subsequent to the last spike before reward delivery and some neurons showed rapid reaction after temporary decrease in firing rates. The activity of these neurons appeared in both FR and VR conditions, and even in the target and the

non-target neurons (Fig. 11, 12, 17 and 18). This result is very interesting since the rats were only required to maintain the firing rates as high as possible during the operant conditioning for faster reward delivery. As described in the Result section, we also checked the acceleration data of the rats in which the characteristic activities mentioned above were observed in order to examine whether these transient changes in neuronal activity were related to actual bodily movements. However, the value of SVM at any sampling time point was not statistically significant. As other studies of operant conditioning of single neurons in primary motor cortex demonstrated similar neuronal activity, i.e., temporary decrease after reward delivery (Fetz and Baker, 1973) or rapid enhancement in non-target neurons (Arduin et al, 2013), the result of the present study is not exceptional. However, there might be some aspects to be tested to precisely understand the activity of neurons observed in the present study. The experimental procedure of the present study, using continuous operation of trials without ITI, was designed to allow the rats to focus on the task as constant as possible. This absence of ITI might have caused somewhat an overlapping problem. Due to the overlapping, every trial was followed immediately by the completion of the previous trial, which means reward, without ITI, making it unclear which phase the neuronal activities represent during the operant conditioning.

4.3. Possible factors that might have affected the result of the present study

4.3.1. The manner of feedback

The result of the present study indicates that, moving to the practical perspective for the development of BMIs, the FR schedule of reinforcement may be more appropriate for training to control external devices using BMIs. The key procedure to progressing with more efficient training is to give the reinforcer immediately after the operant response (immediacy of reinforcement). Neuronal operant conditioning shares this principle with behavioral operant conditioning. Therefore, in order to change the physiological responses and brain activities in a desired direction more

quickly, it is essential to notify those changes using a feedback signal that functions as a conditioned (secondary) reinforcement. In the present study, however, the feedback the rats could evaluate their own performance in real-time was the interval length between reward deliveries. The baseline threshold was set at 20 s and theoretically varied 12 – 28 s in the VR schedule, which might have been too long for the rats to successfully recognize the reinforcement contingency on the neuronal activity enhancement. Therefore, more immediate and frequent feedback signals of enhancement of neuronal activity need to be examined for more effective neuronal operant conditioning.

4.3.2. The manner of threshold setup

In the present study, the number of spikes of individual neurons was counted and used as a source signal for reward delivery. As a result, the mean firing rates of the target neurons were significantly increased across the sessions, reducing the time intervals between reward deliveries. However, the rats could be rewarded only by keeping their target neurons firing constantly without any volitional modulation in their neuronal activity. What the rats were required for rewards was the overall enhancement of firing rates rather than burst-like activity or maintaining firing rates above a specific value. In order to get rewards, the activity patterns of the target neurons had no need to be changed as long as they were spontaneously firing.

If we had set a minimum threshold at a certain level on mean firing rates of the target neurons as Arduin et al. (2013) did and selectively recruited the neuronal activity above the certain mean firing rates for reward delivery, different results might have been obtained.

4.4. Future research

4.4.1. Precise measurement of bodily movements

The on-going progress of research into neuronal operant conditioning confirms the possibility of

volitional enhancement of activity for specific individual neurons. In the present study, we concluded that there was no specific motor activity during the neuronal operant conditioning on the basis of observation by the video camera and detection of fine movements by the three-axis accelerometer. This indicates that motor cortical neurons could be conditioned to increase their firing rates with no increment of bodily movements. The key mechanism to explain such discrepancy of motor cortical activity and bodily movements was the learning-dependent plastic connections between motor cortical neurons and body muscles, formerly suggested in Fetz and Cheney (1987) and Chapin et al. (1999). Also, several former and recent studies (Fetz and Finocchio, 1971; Koralek et al., 2012; Engelhard et al., 2013; Sakurai and Takahashi, 2013) reported the absence of specific body movements or muscle activity during the operant conditioning of neuronal firing.

Although it seems apparent that neuronal activity can be operantly conditioned without body movement and enhanced volitionally by setting direct contingency between changes of neuronal activity and delivery of reward, study on the relationship between bodily movement and neuronal activity should be done in more sophisticated way. Possibility of chance reinforcement of a body movement rather than neuronal activity should always be checked. The question is whether operantly conditioned neuronal firing is directly controlled in certain central pathways alone or through an accidentally reinforced body movement which generates activity in the whole pathways leading to the muscles, including corollary discharge and proprioceptive and sensory feedbacks. In this regard, the bodily movements including speed, direction and muscle tensions of the subjects during neuronal operant conditioning need to be investigated with more precise measurement (Moritz et al., 2008).

4.4.2. Analysis of the activity for neighboring neurons (non-target neurons)

No firing enhancement was observed in any neighboring neurons which were not used for the

operant conditioning. From this finding, we ascertained that neuronal operant conditioning can lead to volitional enhancement of the firing rate in a single target neuron or in a small restricted area close to the target neurons in a motor cortex. However, we also sometimes observed temporary changes in firing patterns for the non-target neurons, despite that the mean firing rates were not significantly changed. From this observation, it needs to record a large number of neurons and analyze the temporary changes in neuronal activity of the neighboring neurons in more detail, besides the averaged amount of their firing. Such analysis will reveal how many individual neurons are modified by neuronal operant conditioning and how differently they are modified in their activity patterns in relation to the target and non-target of conditioning.

4.4.3. Reliability of single neuron's activity as a source of BMI

One serious problem of neural operant conditioning, which uses a small number of neurons for developing high precision BMIs is their limited stability as a source of signals to control a neuroprosthesis. In addition to such a physiological problem, it should be made clear how long conditioned changes of neuronal activity can be retained. This problem is related to one of the major and difficult issues in psychology, i.e., the sustainability of learning, but it should be investigated as it is relevant for long-term reliability of BMIs. Although a previous study (Sakurai and Takahashi, 2013) reported that the conditional enhancement of firing synchrony was retained for more than three days, no experiment of neuronal operant conditioning has examined the limited stability of conditioned changes of neuronal activity. In this regard, transfer of operantly-conditioned firings between different neuronal groups is profitable to compensate the limited stability of source signals and conditioned activity changes. Additional studies in neuroscience (i.e., such as those described in Song et al., 2015) are required to test the possibility of the transfer of conditioned firings in the brain.

Chapter 5. Conclusion

Although the present study mainly describes the learning processes in neuronal operant conditioning with different schedules of reinforcement, changes in firing rate and synchrony of neurons during conditioning should be examined in more detail to observe dynamic changes in neuronal functions in operant conditioning (e.g., Sakurai and Takahashi, 2013).

In the present study, we ascertained that it is possible to cause various changes in activity of individual neurons and small populations of neurons that make up the brain, through operant conditioning of neuronal activity. Such results from neuroscience research as well as discussions of mechanistic and theoretical backgrounds of neural operant conditioning, particularly regarding practical applications of BMI, will lead to the development of clinically significant and highly reliable BMIs using the plastic characteristics of the brain, i.e., recalibrating classifiers of the decoder in BMI (Bishop et al., 2014), and at the same time will contribute to developing methods of rehabilitation that restore or transform the brain functions, i.e., improving the performance of BMI at estimating intention of users (Fan et al., 2014).

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