

**Dissertation**

**Analysis of Rat Hippocampal Local Field  
Potential during Robot Control Task for  
the Development of the Brain-Machine  
Interface**

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Submitted on May 7, 2018

Accepted on August 30, 2018

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## **Abstract**

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The neuroscience studies have brought about a great benefit to human beings. Among them, the brain-machine interface (BMI), or called brain-computer interface (BCI), is one of the breakthroughs and challenging ideas from the viewpoint of engineering and clinical utilities. It has been developed to interpret the neural activities to control a robot, prosthetic device, or computer. In the BMI studies, neural activities in the motor cortex have mainly been considered as the inputs to the external output device. However, it would be better to incorporate the neural information acquired from other brain areas which contain a variety of information other than motor commands. In this study, therefore, I deeply examined the properties of hippocampal theta oscillation during a robot-controlling task in rats which resembles that used in human BMI task. As already known, the hippocampal theta oscillation is involved in a variety of cognitive processes, including the decision-making processes, and changes its parameters prior to the alteration in behavior or motor output, and could provide a clue to differentiate a series of cognitive and behavioral stages consisting of sensory processing, movement preparation, and motor execution. On the other hand, the theta oscillation observed on the scalp of the human frontal midline region (FM theta) is suggested to have similar functional roles in cognitive tasks to those of the rat hippocampal theta. Therefore, it would be useful if an evaluation of the hippocampal or FM theta oscillation is integrated into the BMI to prepare the output device for the incoming motor commands and improve the efficacy of operation of the BMI. For

that purpose, it is crucial to examine what kinds of parameters of the theta oscillation show a change associated with the motor behavior and when the changes occur. In addition, it would also be important to investigate to what degree those changes in oscillatory parameters show a robustness to the difference in the type of motor outputs and cognitive processes.

In the present study, a robot-controlling task by pressing one of the two levers with ipsilateral forelimb was performed. The rat under a mild restrained condition was required to press one of the two levers set in the right and left. The rat had to choose the lever of the same side of the robot location, the correct lever which moved the robot toward the rat and continues to press it several times until the robot arrived at the area within the reach. I found that the relative power of hippocampal theta oscillation, as well as the absolute one, gradually increased during a preparatory period of 6 s before the start of multiple lever pressing regardless of the correctness of lever choice or the side of forelimb usage. There was also a significant difference in theta power after the first choice between correct and incorrect trials: in the correct trials, the theta power was highest during the first lever-releasing period, whereas it was during the second correct lever-pressing period in incorrect trials. I also analyzed the hippocampal theta oscillation around the termination of multiple lever pressing. Irrespective of the side of forelimb usage, the relative power of hippocampal theta oscillation, as well as the absolute one, decreased gradually with the termination of lever pressing to take food from the robot. The dominant frequency of theta oscillation also showed a similar temporal change: the weighted mean frequency of hippocampal theta oscillation increased before the start of multiple lever pressing, irrespective

of the correctness of lever choice or the side of forelimb usage, maintained during multiple lever presses, and then decreased gradually with the termination of lever pressing. There was a significant increase in dominant frequency after the first lever-on (or lever press) in incorrect trials compared to that in the correct trials. I also analyzed the dependency of the theta power and the frequency on the duration of the first lever press. The trial, whose first press lasted less than one second or more than one second, was categorized as an initial-short-press trial or initial-long-press trial, respectively. There was no significant difference between the trial types in both the theta power and frequency. These results suggest that the power and frequency of hippocampal theta oscillation might reflect the similar preparatory and maintaining processes for multiple lever pressing as well as the different aspects of a cognitive process after the first lever-on (or lever press).

Some of these dynamic properties of the rat theta oscillation during the effort-demanding task were similar to those in the monkey FM theta, suggesting that the ideas acquired by examining the usefulness of the hippocampal theta oscillation to improve the BMI might be applicable to human BMI by using FM theta oscillation recorded from the scalp instead of the hippocampal oscillation. Future work should be done to evaluate whether I can improve the performance of BMI by incorporating the information on theta oscillation in addition to that on motor commands recorded from the motor cortex.

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# 1 Introduction

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The hippocampal theta oscillation in animals has been implicated in a variety of cognitive processes (Lever et al., 2014), including the decision-making process (Bett et al., 2012; Bannerman et al., 2012; Taylor et al., 2014), and changes its state prior to the alteration in behavior or motor output. For example, during the serial feature positive discrimination task in eyeblink conditioning, the hippocampal oscillation showed a shift from a large irregular activity to a theta dominant state after the conditional cue light to prepare for the upcoming tone paired with an electrical shock (Tokuda et al., 2014). Also in a jump avoidance task, the amplitude and frequency of hippocampal theta oscillation provided a clue to differentiate a series of cognitive and behavioral stages consisting of sensory processing, movement preparation, and motor execution periods (Bland et al., 2006). This kind of preparatory brain activities will become more important in the tasks that require a larger cognitive demand (Walton et al., 2006). Therefore, it would be useful if an evaluation of the hippocampal oscillation is integrated into the brain-machine interface to prepare the output device for the incoming motor commands. There have been several human studies that analyzed the pre-movement cortical oscillatory activities (Pfurtscheller and Silva, 1999) and utilized the activities to decode the intention of limb movement (López-Larraz et al., 2014; Sburlea et al., 2015).

In the previous work, we reported an attempt to estimate the movement

of an e-pack robot, which carried a small amount of food to the front of a rat, based on the analysis of local field potential (LFP) recorded from the motor areas (Mano et al., 2013). In this task, the rat was trained to press one of the two levers several times with the ipsilateral forelimb to control the robot. Therefore this task is more complicated and more cognitively demanding than a standard operant lever press task, in which the rat presses the lever only once with its dominant forelimb to get a reward. The contribution of hippocampus to this task may be increased with the task difficulty as reported in other tasks, such as delay vs. trace eyeblink conditioning (Beylin et al., 2001), visible vs. hidden platform water maze (Sakimura et al., 1995), and simple vs. visual-discrimination Y-maze (Murray et al., 1999). In fact, it was reported that post-learning inactivation of the hippocampus did not impair the simple light/dark discrimination lever press task, but greatly affected the discrimination of the position of an object on a computer screen (Levcik et al., 2013). Analysis of metabolic activity revealed a participation of the hippocampus to the effort-based decision making in two-lever choice task (Endepols et al., 2010). The participation of the hippocampus in two-lever choice task with a highly cognitive demand is also supported by the lesion experiment, in which the hippocampal lesion had no effect on acquisition of the two-lever choice task or the selective extinction using outcome devaluation protocol, but impaired the extinction by degradation of the contingency between the lever press and the reward (Corbit and Balleine, 2000). Accordingly, several types of two-lever choice task have been used to investigate the relationship among the higher cognitive systems (Mishima et al., 2002; Floresco et al., 2008). In addition, the selective use of right or left forelimb ipsilateral to the robot position



might also elevate the cognitive demand, because rats show a handedness, having a preference to use the right forepaw (GÜVEN et al., 2003) and reversal of handedness activated the hippocampus (Cupello and Hyden, 1978).

In the present study, I deeply investigated the hippocampal theta oscillation during the bimanual two-lever choice task for robot control, focusing on the preparatory phase. I found that the relative power and dominant frequency of theta oscillation increased before the start of multiple lever pressing, irrespective of the correctness of choice or the side of forelimb usage, suggesting a participation of hippocampal theta oscillation in a preparatory process for multiple lever pressing but not in the cognitive process for the correct side or the detail of forelimb control.

## **2 Material and Method**

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### **2.1 *Animals and ethics statement***

The animals used in this research were 10 week-old Wistar/ST male rats at the start of the experiment ( $n = 5$ , Japan SLC, Inc., Hamamatsu, Shizuoka, Japan), and housed individually in standard plastic cages on a 12/12 h light/dark cycle with free access to drinking water. They were habituated to handling and their body weight was gradually reduced to about 85% over a week before the training with food rewards started. All of the experimental procedure was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Experimental Animal Committee of the University of Toyama (A2014ENG-7). Throughout the experiment, all efforts were made to minimize the use of animals and to optimize their comfort.

### **2.2 *Conditioning procedures***

The rats were trained with a food reward in three progressive stages to press a correct lever to move a food-carrying robot towards them, which took about three to four weeks in total. A daily session contained approximately more than 20 trials, in each of which the rat got a small amount of food. A daily session was terminated if the rat stopped to press the lever or the session exceeded 1 hr.

At the end of the daily session, they were fed with additional food to maintain the body weight. In the first stage, a rat was placed in a transparent plastic cage (28×45×20 cm) equipped with two micro-switch levers, whose tips were set at 0.5 cm height and 7.5 cm apart horizontally, and two green light-emitting diodes (LED) set above the lever tips at 3.2 cm height. One of the LEDs was manually turned on at a time (Fig. 1A). The rat was allowed to use either forelimb to press the correct lever beneath the illuminating LED to get a food pellet. The side of illuminating LED was changed from session to session in such a way that the same side was not used in more than two consecutive sessions and the total number of sessions was almost balanced between the right and the left side. The rats were trained until they achieved more than 90% correct for both sides, calculated by dividing the number of correct lever presses by the total number of lever presses in a session. In the second stage of training, they were habituated to take food under a mild restrained condition with a neck collar and an acrylic cylinder to limit the body movement and the forelimb usage (Fig. 1B). After two days of habituation, they were trained to press the lever ipsilateral to the position of an e-puck robot (Cyberbotics Ltd., Switzerland) using only the ipsilateral forelimb. The robot had a food handler and a green LED, illuminated constantly during a daily session. The robot was placed either on the right or left start point on a U-shaped track, about 13 cm away from hand reach of the rat, and moved towards or away from the rat along the track when the ipsilateral correct lever or the contralateral incorrect lever, respectively, was pressed (Mano et al., 2013). The micro-switch levers were connected through a serial interface to the computer, which controlled the e-puck robot via a Bluetooth interface.

The side of start point was identical throughout the trials in a daily session and was changed randomly from session to session as in the first stage. A trial was categorized as correct if the first and the subsequent lever presses were ipsilateral to the robot. In incorrect trials, the first press was wrong but most of the subsequent ones were correct. The rats were trained until they achieved more than 90% correct for both sides, calculated by dividing the number of correct trials by the total number of trials. In the last stage, the side of the start point was changed randomly within a session and the rats have trained accordingly as described above.

### **2.3    *Surgery***

After reaching 90 % correct in the third stage, the rats received a surgery for setting the electrodes to record the hippocampal oscillation. The electrode was constructed from a pair of twisted Teflon-coated stainless steel wires (140  $\mu\text{m}$  in diameter, A-M Systems, Sequim, WA, USA), and implanted bilaterally in the dorsal hippocampus under anesthesia with sodium pentobarbital (65 mg/kg i.p., Kyoritsu Seiyaku, Tokyo, Japan). Isoflurane (1–2%, Abbot Japan, Osaka) was also used when necessary. The coordinates of the electrode were 4.8 mm antero-posteriorly, 3.2 mm laterally, and 2.2 mm dorsoventrally from bregma, according to the standard brain atlas (Paxinos and Watson, 1998). The final depth of electrode was decided based on the LFP profile recorded during the implantation. After the surgery, the animals were injected with ampicillin (100 mg/kg i.p., Meiji Seika, Tokyo, Japan) and warmed until they moved spontaneously. During

the recovery period, rats were fed wet paste food instead of standard pellet food.

## **2.4 *Analysis of the hippocampal oscillation***

After a week of recovery, the same conditioning as in the third stage restarted. The hippocampal LFP was recorded using the Cheetah system (Neuralynx, Tucson, AZ, USA). The hippocampal LFP was band-pass filtered between 1 and 475 Hz and sampled at 7,575 Hz, which was reduced off-line to 1,515 Hz before further analyses. The data collected from three sessions in each rat were analyzed with custom-written programs on MATLAB (The MathWorks, Natick, MA, USA).

To analyze the theta oscillation before and after the multiple lever pressing (Figs. 3, 4, 5, 6), I used the multi-taper FFT MATLAB package written by Mitra and Pesaran (1999), with a frequency range of 130 Hz, a time-window of 1 s, and a stepping width of 0.2 s. The relative power of theta frequency band (6–9 Hz) divided by the power of total band (1–30 Hz), the absolute power expressed as a fractional change of that 10 s before the first lever press or that at the end of the last lever press, and the weighted-average frequency of theta band (4–9 Hz) were calculated. These analyzed data during a session were grouped into correct trials and incorrect trials. The data from correct trials were further divided into the right- and left-forelimb trials. I also analyzed the difference in the theta power and frequency between the trials that differed in the duration of the first lever press: the initial-short-press trial whose duration of the first press lasted less than one second and the initial-long-press trial with a longer duration of the first press (Fig. 9).

To analyze the change in theta power during the first and second lever presses as well as between them (Fig. 7B), I calculated the power spectral density after matching the frequency resolution, which varied due to the difference in data length. The power spectral density at each frequency was normalized by the total amount of those between 1 and 30 Hz during the first lever press and then averaged over the trials and animals.

To consider the phase lock to the behavior, I calculated the theta phase at the timing of the first lever press and the last lever release in the right- and left-forelimb trials. The peak and trough of the theta oscillation around the lever press were extracted from the data. Then, the theta phase at the lever press was determined by a linear interpolation between the peak and the trough; the theta peak was assigned as phase zero and theta troughs as phase  $\pi$ . The significance of the resultant theta phase distribution was confirmed with the Rayleigh test for circular uniformity.

## **2.5 *Histology***

After completion of the behavioral experiment, a current of 50  $\mu$ A was passed for 45 s between the electrode tips under anesthesia with sodium pentobarbital (65 mg/kg i.p.) to confirm their location. After several days, the rats were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg i.p., Kyoritsu Seiyaku, Tokyo, Japan) and perfused transcardially with 0.9% saline, followed by 10% formalin. The brains were taken out, stored in 10% formalin, and then sectioned at 40  $\mu$ m on a cryostat. The brain slices were mounted on glass slides

and Nissle stained to examine the electrode placement under a microscope.

## **2.6 *Statistical analyses***

Statistical analyses were conducted using the statistical software (SPSS, Chicago, IL, USA). Data were expressed as mean  $\pm$  standard error of the mean. I used the one-way repeated measures ANOVA or the two-way repeated measures ANOVA. In addition, I also use the paired t-test or the pairwise comparison for post hoc test after the ANOVA. The difference was considered as statistically significant when the p-value was less than 0.05.

### 3 Result

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During the recording sessions, per trial, the rats pressed the lever an average of  $2.76 \pm 0.33$  times per trial. In addition, the rats performed  $24.8 \pm 2.7$  trials per session. The inter-trial interval was  $44.2 \pm 3.3$  s during a session. All the electrodes were placed in the dorsal hippocampus (Fig. 2A) and the power spectrum of the hippocampal LFP calculated over a conditioning session demonstrated a clear peak in the theta range (Fig. 2C). The rats showed a good performance throughout the recording sessions, during which the percentage of correct lever press was  $86.4 \pm 4.4\%$ . To elucidate the relationships between the change in the hippocampal LFP and the behavioral states, I separately analyzed the LFPs during the following time periods: (i) before the first lever press, (ii) during the initial lever presses, and (iii) after the termination of multiple lever presses.

#### 3.1 *Spectrogram before the first lever press in the correct and incorrect trials*

The spectrogram analysis of the hippocampal LFP before the first lever press revealed a gradual increase in the relative power of theta frequency range (6–9 Hz) in the correct trials (Fig. 3Aa) as well as in the incorrect trials (Fig. 3Ab). As shown, the increase started about 6 s before the first lever press. Analysis of the absolute power of LFP revealed that the gradual increase in the relative theta power resulted from both the increase in the absolute power of theta frequency



range and the decrease in that of the other frequency ranges (Figs. 3Ac and 3Ad).

To quantitatively compare the time course between the correct and the incorrect trials, I calculated the power of 6–9 Hz of the theta band. Figure 3Ba shows the change in relative power of the theta band. Two-way repeated measures analysis of variance (ANOVA) did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 1.334$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 2.868$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 34.689$ ,  $p < 10^{-5}$ ). The absolute power of theta band also showed a similar change to that of the relative power (Fig. 3Bb), confirming that the theta power actually increased before the first lever press in the correct trials as well as in the incorrect trials. Two-way ANOVA with repeated measures did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 1.578$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.168$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 3.766$ ,  $p < 0.05$ ).

### ***3.2 Spectrogram before the first lever press in the right- and left-forelimb trials***

I further analyzed the gradual increase in the relative and absolute theta powers before the first lever press by separating the correct trials to those using the right or left forelimbs (Figs 4A and B). As shown, the increase was almost similar between the right-forelimb trials and left-forelimb trials. Two-way ANOVA with repeated measures applied to the relative theta power did not show a significant

effect in the interaction between time and trial type ( $F_{(46,184)} = 0.833$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.059$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 34.491$ ,  $p < 10^{-5}$ ). Similarly in the absolute theta power, there was no significant effect in the interaction between time and trial type ( $F_{(46,184)} = 1.341$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.047$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 12.916$ ,  $p < 10^{-5}$ ).

### ***3.3 Spectrogram before the first lever press in the right and left robot-presentation***

I further considered the effect of the difference of the robot location on the hippocampal oscillation to evaluate the visually guided spatial cognition or perspective. The gradual increase in the relative and absolute theta powers before the first lever press have been observed both right and left robot presentation trials (Figs 5A and B). As shown, the increase was almost similar between the right and left presentation trials. Two-way ANOVA with repeated measures applied to the relative theta power did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 0.267$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.098$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 46.532$ ,  $p < 10^{-5}$ ). Similarly in the absolute theta power, there was no significant effect in the interaction between time and trial type ( $F_{(46,184)} = 0.816$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 5.124$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 11.553$ ,  $p < 10^{-5}$ ).

### 3.4 *Spectrogram after the termination of the multiple lever presses using the right or left forelimb*

Analysis of the spectrogram of hippocampal LFP during a period after termination of the lever presses, the period when the rats took the food with their hands and ate it, revealed that the elevated relative theta power associated with multiple lever press gradually decreased to the basal level 8–9 s after the last lever release both in the right- and left-forelimb trials (Fig. 6Aa and 6Ab, respectively). Analysis of the absolute power of LFP revealed that the gradual decrease in the relative theta power resulted from both the decrease in the absolute power of theta frequency range and increase in that of the other frequency ranges (Figs. 6Ac and 6Ad).

To quantitatively compare the time course between the right- and the left-forelimb trials, I calculated the power of 6–9 Hz of the theta band. Figure 6Ba shows the change in relative power of the theta band. Two-way ANOVA with repeated measures did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 0.891$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.446$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 16.063$ ,  $p < 10^{-5}$ ). The absolute power of theta band also showed a similar but somewhat earlier decrease compared to that of the relative power (Fig. 6Bb), confirming that the theta power actually decreased with the termination of multiple lever pressing in the right-forelimb trials as well as in the left-forelimb trials. Two-way ANOVA with repeated measures did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 0.943$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.495$ ,

$p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 1.629$ ,  $p < 0.05$ ).

### ***3.5 Spectral difference between the correct and incorrect trials during the first and the second lever presses***

The critical behavioral difference between the correct and incorrect trials was that the first lever press was wrong but the second and subsequent lever presses were correct in the incorrect trials, while the entire lever presses were correct in the correct trials. It means that the second lever press was the first correct lever press in the incorrect trials. Therefore, I focused on the difference in the power spectrum during the first and the second lever presses. Before examining the spectral difference, I checked behavioral difference by comparing the duration of the first and the second lever presses as well as the lever release between them (Fig. 7A). The duration of lever release after the first incorrect lever press in the incorrect trials was longer than that after the first correct lever press in the correct trials, suggesting that the rat showed a pause period before they made a shift from the incorrect choice to the correct choice. Two-way ANOVA with repeated measures did not show a significant effect in the interaction between trial type and lever press/release ( $F_{(2,8)} = 4.393$ ,  $p = 0.051$ ) or in lever press/release ( $F_{(2,8)} = 4.419$ ,  $p = 0.051$ ), but there was a significant effect in trial type ( $F_{(1,4)} = 9.1594$ ,  $p < 0.05$ ).

Then, I investigated the change in power spectrum during the behavioral sequence from the first to the second lever press and found a difference between the correct and incorrect trials (Fig. 7B). In the correct trials, the theta power

increased after the release of the first correct lever and then decreased to the level of the first lever press (Fig. 7Ba). In contrast in the incorrect trials, it did not so much increase after the release of the first incorrect lever press but increased largely during the second correct lever pressing (Fig. 7Bb). To quantitatively analyze the change in theta power during the behavioral sequence from the first to the second lever press, I calculated the power of 6–9 Hz of the theta band, normalized by the total power (1–30 Hz) of the first lever press in each trial type (Fig. 7C). One-way repeated measures ANOVA indicated a significant effect both in the correct trial ( $F_{(2,8)} = 41.917$ ,  $p < 10^{-5}$ ) as well as in the incorrect trial ( $F_{(2,8)} = 5.106$ ,  $p < 0.05$ ). The additional analysis using paired-samples  $t$ -test confirmed significant differences between the first press and the first release ( $p < 0.01$ ), the first release and the second press ( $p < 0.05$ ) and the second press and the first press ( $p < 0.05$ ) in the correct trial, whereas a significant difference was only observed between the first press and the second press ( $p < 0.05$ ) in the incorrect trial.

### ***3.6 Comparison of the change in theta frequency between the correct and incorrect trials and between the right- and left-forelimb trials***

In addition to the theta power, I examined the change in theta frequency associated with the multiple lever pressing. The weighted average frequency of theta oscillation started to increase about 6 s before the first lever press both in the correct and incorrect trials (Fig. 8A). Two-way ANOVA with repeated measures

did not show a significant effect in the interaction between time and trial type ( $F_{(9,36)} = 1.03$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.546$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(9,36)} = 6.965$ ,  $p < 10^{-5}$ ). When the correct trials were divided to the right- and left-forelimb trials before the first lever press (Fig. 8Ba), there was also no significant effect in the interaction between time and trial type ( $F_{(9,36)} = 1.039$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 1.622$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(9,36)} = 17.873$ ,  $p < 10^{-5}$ ). This tendency of similarity in the change of dominant frequency between the right- and left-forelimb trials was almost the same for the data after the last lever press. The increased dominant frequency of theta oscillation was lowered after the termination of multiple lever pressing both in the right- and left-forelimb correct trials (Fig. 8Bb). It should be noted that the dominant theta frequency began to decrease before the release of the last lever and reached to the basal level after 3–4 s, much earlier than the decrease in the relative theta power (Fig. 6Ba) and seemed in parallel with that in the absolute theta power (Fig. 6Bb). Two-way ANOVA with repeated measures, applied to the data including those 2 s before the last lever release, did not show a significant effect in the interaction between time and trial type ( $F_{(11,44)} = 0.599$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(11,44)} = 5.848$ ,  $p < 10^{-5}$ ) and marginally in trial type ( $F_{(1,4)} = 7.889$ ,  $p < 0.05$  ( $= 0.048$ )). The pairwise comparison showed a significant difference between the data at -2 s and those after 2 s ( $p < 0.05$ ) but not between the data at 2 s and those after 2 s, indicating that the dominant frequency returned to the basal level in about 2 s.

### ***3.7 The change in the relative theta power and the frequency in different ways of lever pressing***

There were no clear difference in the prior increase of theta power and frequency on the correctness of the lever press, the usage of handedness and the position of robot-presentation (Fig. 3Ba, 4Ba, 5Ba, 8A). I also investigated the dependence of the theta power and the frequency on the duration of the first lever-press. On the duration of the first lever press, at least, there is two type of lever press: many times of lever presses or just a few lever press within a trial (Fig. 9Aa and 9Ab). The former is the initial-short-press trial, and the later is the initial-long-press trial. To examine whether the multiple lever-pressing results from the low cognitive processing, I compared the latencies as well as the hippocampal theta power, and frequencies in the first lever press between the trials. Although there are positive tendency that the lever press which seems to contain the thought shows the longer latency before lever press, the latencies to the first lever press of multiple pressing have no significant difference between the initial-short-press and the initial-long-press trials (Fig. 9B). The relative theta power similarly increased in both the trial types (Fig. 9C), as in the case of the correctness of the lever press and the usage of handedness. Two-way ANOVA with repeated measures applied to the relative theta power did not show a significant effect in the interaction between time and trial type ( $F_{(46,184)} = 0.833$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.059$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(46,184)} = 34.491$ ,  $p < 10^{-5}$ ). Similarly, two-way ANOVA with repeated measure applied to the theta frequency did not show a significant effect in the interaction

between time and trial type ( $F_{(9,36)} = 1.03$ ,  $p > 0.05$ ) or in trial type ( $F_{(1,4)} = 0.546$ ,  $p > 0.05$ ), but there was a significant effect in time ( $F_{(9,36)} = 6.965$ ,  $p < 10^{-5}$ ) (Fig. 9D).

### ***3.8 Theta phase around lever press in right- and left-forelimb***

I also examined the behavioral coupling to the theta phase in handedness. To evaluate the statistical significance ( $p < 0.05$ ) on the theta phase, the phase value are considered with the Rayleigh test, which is a test for uniform circular distributions. Across the three sessions in each rat, the theta-phase distribution has no significant uniformity in the first lever on ( $p > 0.05$ ) and last lever off ( $p > 0.05$ ) between right- and left-forelimb trials (Fig. 10).



## 4 Discussion

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In the present work, I investigated the effects of different forelimb usage and correctness of lever choice on the hippocampal oscillation during the preparatory phase for lever selection. I found that both the power and frequency of theta oscillation increased before the start of lever pressing irrespective of the correct/incorrect lever choice or the right/left hand usage, suggesting that these parameters might reflect a general preparatory process like an attentional process for multiple lever pressing and could be used as a triggering cue for the BMI to start the processing of brain activities for controlling the output devices.

### 4.1 *Prior increase in the power of hippocampal theta oscillation*

Whishaw and Vanderwolf (1973) reported that the theta oscillation was observed in head turning and lever pressing, although it was much smaller than in large movements such as running and jumping (Whishaw and Vanderwolf, 1973). Buño and Velluti (1977) also reported that ongoing theta increased in amplitude and frequency 1 s before and after pressing in the initial stage of sessions (Buño and Velluti, 1977). Consistent with this, I found that the theta power began to rise about 5–6 s before initial lever press, remained high during a sequence of multiple lever presses, and decreased gradually after they stopped lever pressing to get the food (Figs. 3–6). Because the theta power remained high during the sequence

of the lever on and off within the multiple lever presses, the change in theta power may not directly be coupled to the motor output, but be involved in some cognitive processes. The long latency of 5–6 s from the initiation of theta power increase to the start of lever pressing as well as the gradual decrease lasting 5–6 s after the termination of lever pressing also supports this view. Interestingly in a conditional visual discrimination task (Wyble et al., 2004), the prior change in hippocampal theta power was different between the levers with distinct roles: the theta power increased 1 s before pressing the center lever that initiated a trial of the operant task, while it decreased largely before pressing one of the two lateral levers for correct choice. In addition, the increase prior to the trial initiation was observed only during the initial stage of training, whereas the decrease before bar selection appeared even in the late well-learned stage. This lever selection between the lateral bars is similar to that made in the initial lever press in my experiment, although the direction of change in theta power was opposite. These results suggest that the prior change in hippocampal theta power before lever press might be involved in several cognitive processes and depend on the task paradigm as well as on the functional role of the lever assigned in the task.

I also found that this increase in theta power did not depend on the correctness of lever choice, suggesting that the prior increase in theta power might be associated with the start of lever pressing itself. This non-dependency on the correctness is different from the findings in the negative patterning task, in which the relative theta power began to increase just before the correct lever press and diminished within 1 s only in the reinforced trials, but not before the incorrect lever press in the non-reinforced trials (Sakimoto et al., 2013), suggesting that the

power increase just before the lever press might depend on the correct cognition of the trial type in the negative patterning task. This is consistent with the findings during the decision-making period in the four-arm radial maze, in which theta activity was elevated before correct choices but not before incorrect ones (Belchior et al., 2014). In contrast in the present work, the power increase starting long before the lever press might not be related to such kinds of cognitive aspects, because it was almost similar between the correct and incorrect lever press as well as between the right and left trials.

In addition, I found that the prior increase in theta power did not depend on the side of forelimb usage, suggesting that it did not directly couple to control of the contralateral forepaw movement. In the forelimb motor cortex, the theta power showed significant increases when the contralateral dominant hand was used during the food-reaching task (Vyazovskiy and Tobler, 2008). Indirect coupling of the increase in hippocampal theta power to the contralateral forepaw control was also suggested by the long interval of 6 s between the initiation of theta increase and the start of lever pressing (Fig. 4). Ohishi et al. (2003) reported that the evoked potential recorded in the contralateral forelimb motor cortex started 0.5–1.5 s before self-paced lever pressing and was eliminated by the ipsilateral hemicerebellectomy (Ohishi et al., 2003), suggesting that the pre-movement potential directly coupled to the control of contralateral forelimb starts around 1 s before the lever press, much shorter than the start of an increase in the hippocampal theta power in the present study.

Overall, the prior increase in the power of hippocampal theta oscillation in

the present work may not be involved in the preprocessing of forelimb control or the cognition of trial type but may represent the intention to start the multiple lever pressing.

## **4.2 *Prior increase in the hippocampal theta frequency***

In addition to the change in theta power, the dominant theta frequency started to increase about 5–6 s before the first lever-press in the session, tended to decrease but kept high during multiple pressing, and then returned to the preparatory level after the termination of lever pressing (Fig. 8), suggesting no direct coupling to each lever on and off. This increase in frequency did not depend either on the correctness of the lever choice (Fig. 8A) or the side of forelimb usage (Fig. 8B). Therefore, the change in theta frequency almost paralleled that in theta power both during the preparatory phase as well as the execution phase.

Simultaneous change in frequency and power of hippocampal theta oscillation, with a positive or negative correlation between them, has been reported in several types of behavior with large body movements (Bland et al., 2006; Whishaw and Vanderwolf, 1973; Belchior et al., 2014) as well as in the lever-pressing task (Buño and Velluti, 1977). For example in the jump avoidance task, the theta frequency increased while the amplitude decreased, showing a negative correlation, during the preparatory period of 0.5–1 s before jump, whereas during the execution of jumping both the frequency and power increased rapidly and then decreased, showing a positive correlation (Bland et al., 2006). In the lever-pressing task, the amplitude and frequency of theta oscillation increased 1

s before and after the lever press, showing a positive correlation (Buño and Veluti, 1977). Thus, the frequency of theta oscillation might not always change in parallel with the power of theta oscillation but depend on the type of movement and cognitive demand, suggesting that they have the closely coupled but different roles in preparing and executing motor behaviors.

### ***4.3 Subsequent increase in the theta power after the first lever press***

Although the prior increase in theta power was indistinguishable between the correct and incorrect trials, there was a significant difference in the change of theta power after the first lever choice: the theta power in the correct trials increased further after the first correct lever off and then decreased during the second correct lever on, whereas the theta power in the incorrect trials did not show a large increase after the first incorrect lever off, instead it increased greatly during the second correct lever on (Figs. 7B, 7C). Therefore, the change in theta power after the start of multiple pressing might reflect the change in cognition of correctness of the lever press made by the animal. Overall, the increase in theta power might involve both the attention- or decision-related gradual activities before the first lever press and the transient cognition-related activities on the first correct choice. Similar two-phase increase in theta power was recorded from the medial prefrontal cortex and the rostral anterior cingulate cortex in the self-initiated hand-movement task with a waiting period, in which the monkeys were trained to release the lever after holding it in a resting position for more than 6

s (Tsujimoto et al., 2006). It was found that the theta power increased gradually 3–4 s before releasing the lever both in the rewarded and unrewarded trials and showed the second increase after the reward delivery in the rewarded trials or a decrease in the unrewarded trials. Although the second transient peak might involve the response to the reward, it was suggested to include the process of success/error judgment (Tsujimoto et al., 2006). Because in the present work the rats were not rewarded until the robot came within their reach, the increase in theta power after the correct lever press might reflect the success/error judgment at that point rather than the rewarded response

#### ***4.4 Potential use of the prior and subsequent changes in hippocampal oscillation for BMI***

There have been a lot of studies in humans that demonstrated the cortical potentials preceding self-initiated movements, which might be used for BMI. For example, it was shown that there were distinct patterns of cortical activity before the start of different self-initiated arm movements in healthy subjects as well as in patients suffering spinal cord injury, demonstrating that the intention of self-initiated limb movements could be detected using the pre-movement cortical signals (López-Larraz et al., 2014). The intention to walk could also be detected by monitoring the pre-movement cortical potential in stroke patients (Sburlea et al., 2015). However, the change in cortical oscillation observed in these human studies was a decrease in the power of frequencies in the range of  $\alpha$  and  $\beta$  bands (7–30 Hz), termed the event-related desynchronization, which started about 1 s

before the movement and sustained during the movement. In contrast in the present animal study, the increase in hippocampal theta oscillation did not show the laterality and started 5–6 s before the lever press, suggesting an intentional process that could be detected from brain oscillatory activities prior to the cortical desynchronization. The present results would provide an additional possible source to improve the BMI, which could prepare the output device for the incoming motor commands. In addition, the subsequent change in theta power after motor execution might also be useful to improve the BMI. If the post-execution increase in theta power, which contains information about the success/error judgment by the subject, is fed back to the BMI, it can act as an error-teaching signal to cause the BMI to learn to appropriately interpret the cortical signals to produce correct motor commands, resulting in a gradual improvement of the BMI performance.

Although direct recording from the hippocampus would not be plausible in humans, the theta-band oscillation recorded from the frontal midline region of the scalp, called FM theta, will be one of the alternative candidates. The human FM theta has been implicated in several mental operations, including working memory and attentional processes (Inanaga, K, 1998; Mitchell et al., 2008). Although the functional and pharmacological characteristics are not always the same between the hippocampal theta and the FM theta (Mitchell et al., 2008), the FM theta showed similar changes during the hand-movement task in monkeys (Tsujimoto et al., 2006) to those in rat hippocampal theta in the present study. Therefore, the FM theta might be useful to improve the BMI by combining with other cortical activities.

## 5 Conclusions

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In the bimanual two-lever choice task with multiple pressing, both the power and frequency of hippocampal theta oscillation increased 5–6 s before the first lever press, irrespective of the correctness of the first choice or the usage of the right/left forelimb. In addition, there was a significant increase in the theta power after the first correct lever press. These results suggest that the change in power and frequency of theta oscillation may reflect a preparatory process for lever pressing as well as cognitive processes after the lever press, both of which could be used to improve the BMI.



## 6 Acknowledgements

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I would like to express my gratitude to my supervisor, Professor Shigenori Kawahara, for providing me with a precious opportunity to study as a Ph.D. candidate in his laboratory. I would not be possible to write this thesis without his invaluable discussion, untiring dedication, and considerate encouragement.

I am extremely grateful to Professor Genci Capi for giving me the chance to collaborate with different study region. He also contributed to my experiment to construct task environment.

I also would like to express my sincere appreciation to the other members, Dr. Md. Ashrafur Rahman, Dr. Ryota Miyata, Dr. Endri Rama, and Mr. Katsunari Sano. Dr. Rahaman and Dr. Rama inspired me to discuss scientific issues with English communication. They also inspired me not only as a collaborator but also as one of a friend. I believe that making a good relationship with an international student would be an invaluable experience for my future life. Dr. Miyata helped me a lot not only in my data analysis but also as one of senior to share my private issue. He made so many times for me to discuss methodology in my experiment and its mathematical background deeply in Skype. Mr. Sano is one of my junior fellows. He truly contributed to my experiment and shared his skill to go ahead with my experiment.

Finally, I want to dedicate my sincere thanks to my dear parents. I was fortunate to be able to get their understanding, patience, and support during

the entire period of my study. I could not complete this thesis without their dedication.

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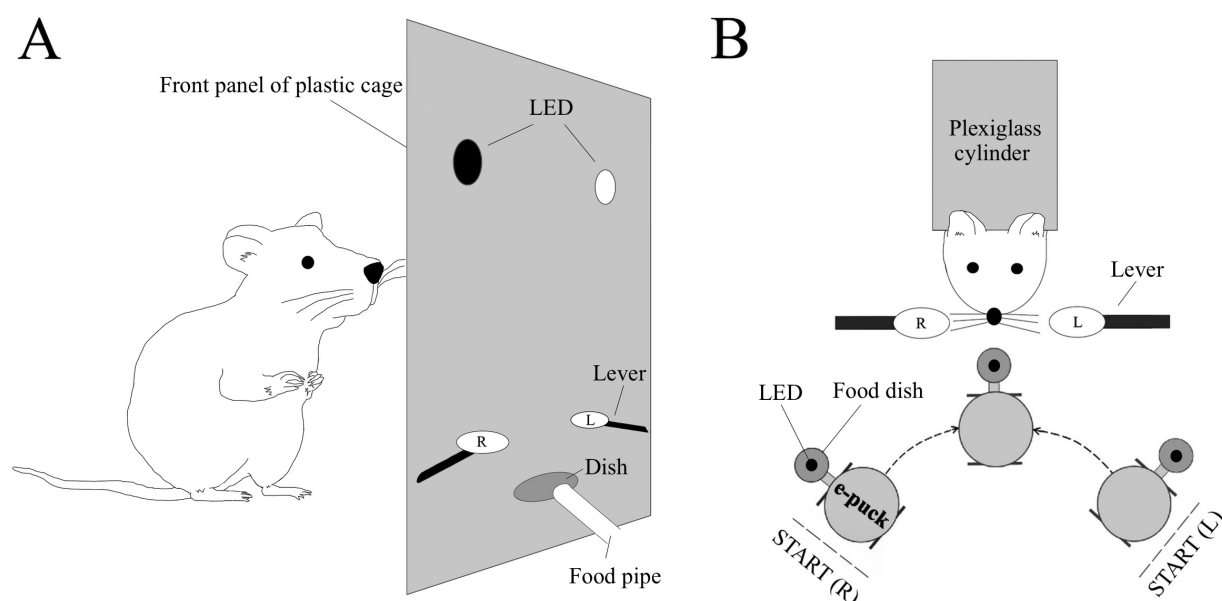
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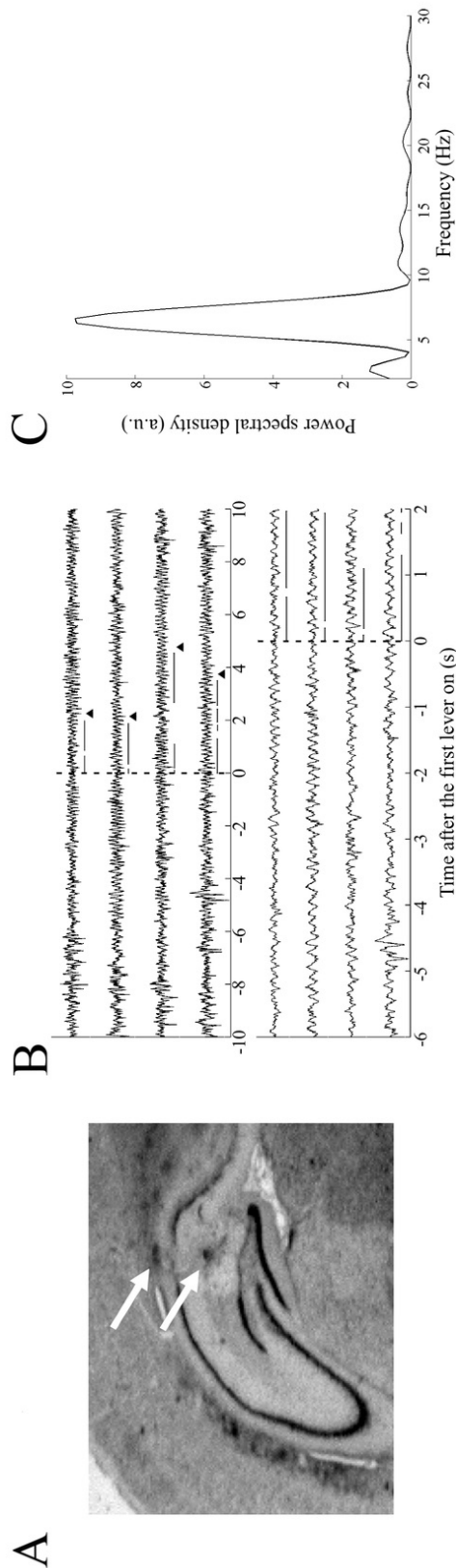
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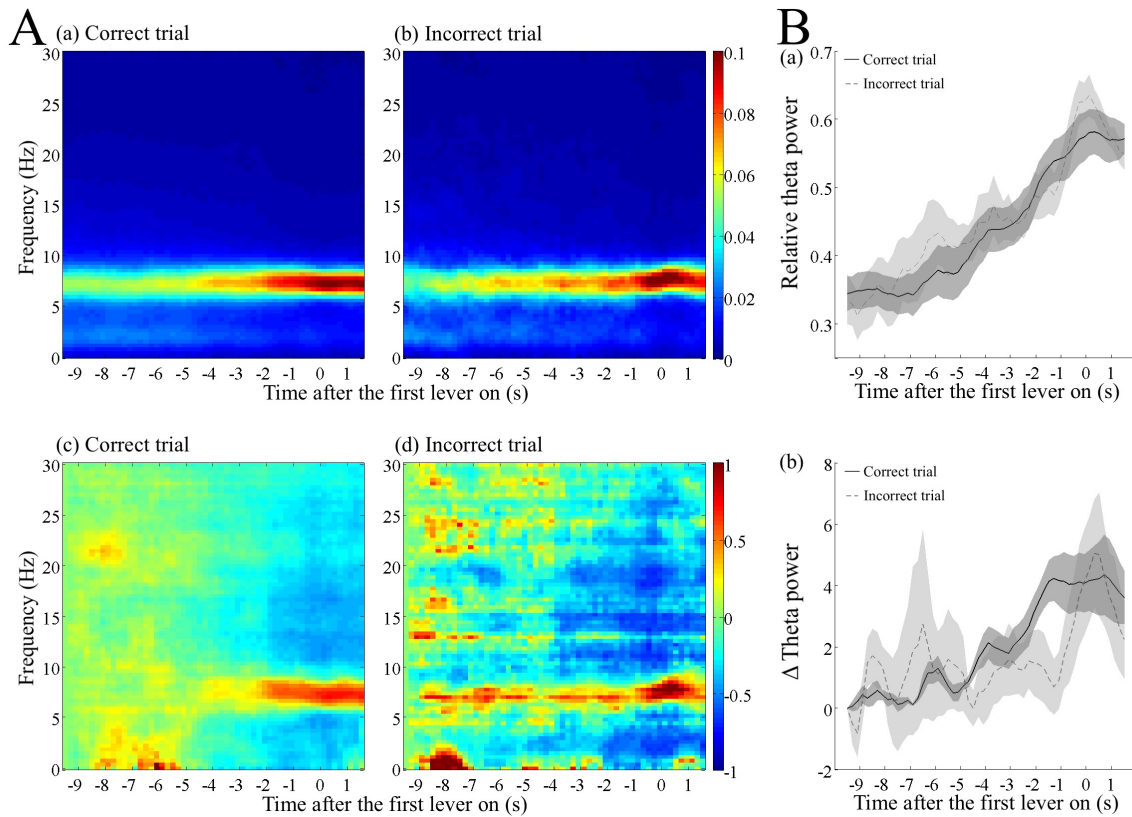




**Fig. 1. Conditioning apparatuses.** (A) Schematic diagram of the operant lever press task in the first stage of conditioning (side view). Rats were trained to press one of the levers ipsilateral to the illuminating LED to get food. The right (R) and left (L) levers were placed in the conditioning box and attached to micro-switches at their bases. (B) Schematic diagram of the robot controlling task in the second and the third stages of conditioning (top view). Restrained rats were trained to press several times the correct lever ipsilateral to the robot until the robot came to the area within their reach. The robot had a dish with food and an illuminating LED

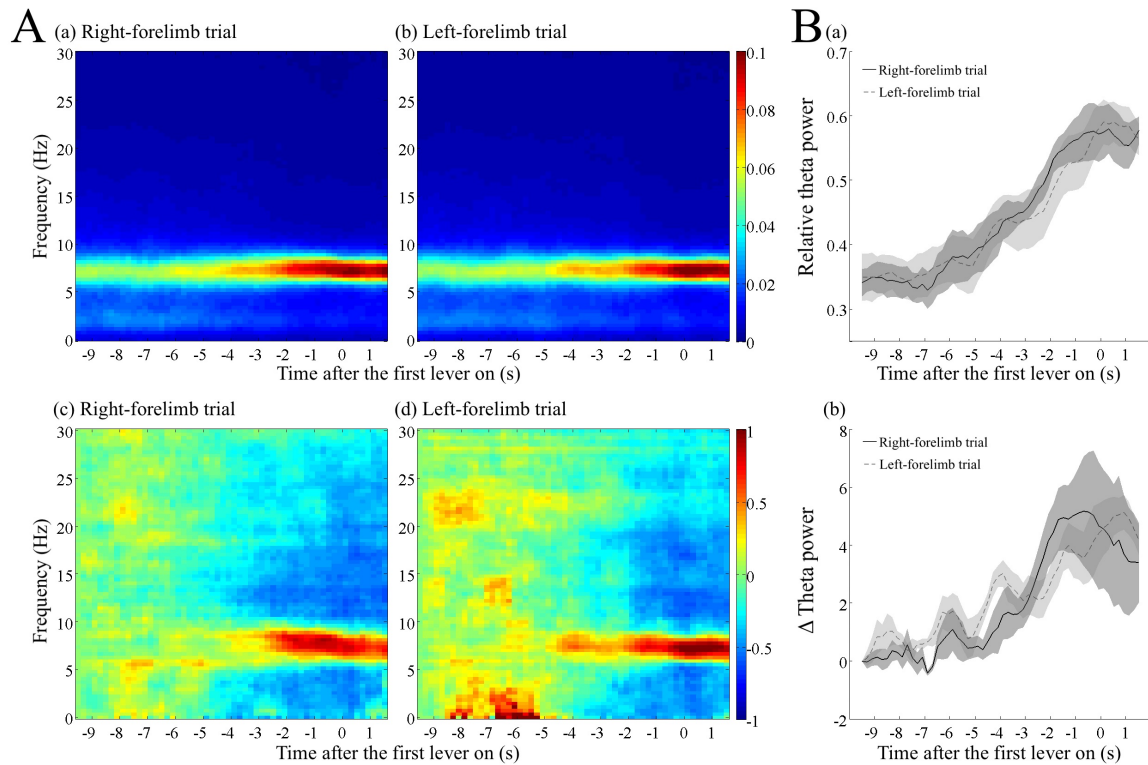


**Fig. 2. Electrodeposition and the hippocampal local field potential (LFP).** (A) Typical electrodeposition in the dorsal hippocampus. The arrows indicate the tips of the twisted electrode, which were marked electrically after the completion of the behavioral experiment. (B) Typical hippocampal LFP data around the lever press. The LFP data in four trials are shown on a longer and shorter timescale in upper and lower traces, respectively. The times before the first lever press are indicated as negative values. Horizontal lines under each LFP trace indicate the lever presses. The rat took a food from the robot after the final lever off, indicated by arrowheads in the upper traces. (C) Typical power spectral density of the hippocampal LFP during a session plotted in arbitrary units.

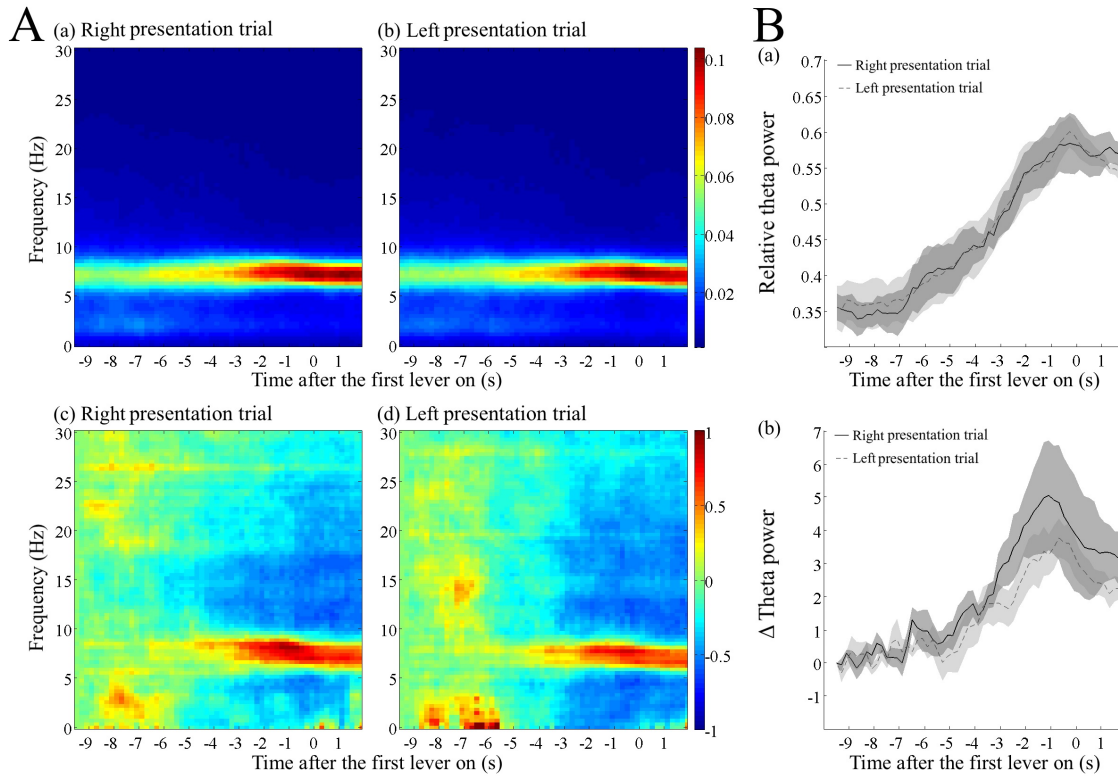


**Fig. 3. Increase in the relative and absolute theta power before the first lever press in the correct and incorrect trials.** (A) Dynamic power spectra in the correct (a, c) and incorrect trials (b, d). A time window of 1 s and a stepping width of 0.2 s were used. The frequency resolution was about 0.36 Hz. The data were averaged over the trial type in each rat and then overall the rats. The relative power divided by the total power of 1–30 Hz (a, b) and the absolute power subtracted and normalized by that 10 s before the first lever press (c, d) were calculated for each frequency. The times before the first lever press are indicated as negative values. The pseudocolor scales in the right indicate the relative power and the normalized absolute power. (B) Increase in the relative (a) and the normalized absolute (b) power of the theta frequency band. The power data of each frequency in A were integrated over the theta frequency band of

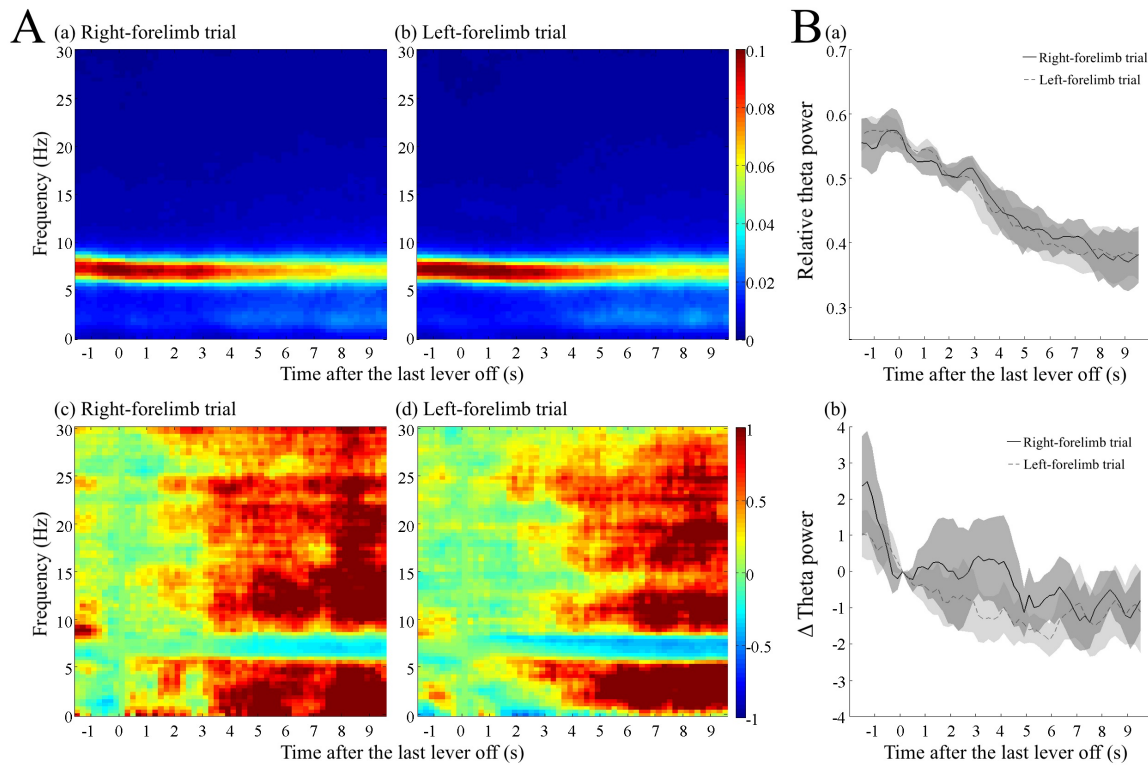
6–9 Hz. The black and dotted lines indicate the data averaged over the correct and incorrect trials in each rat, respectively, and then over the rats. The shaded areas associated with the lines are standard error of the mean.



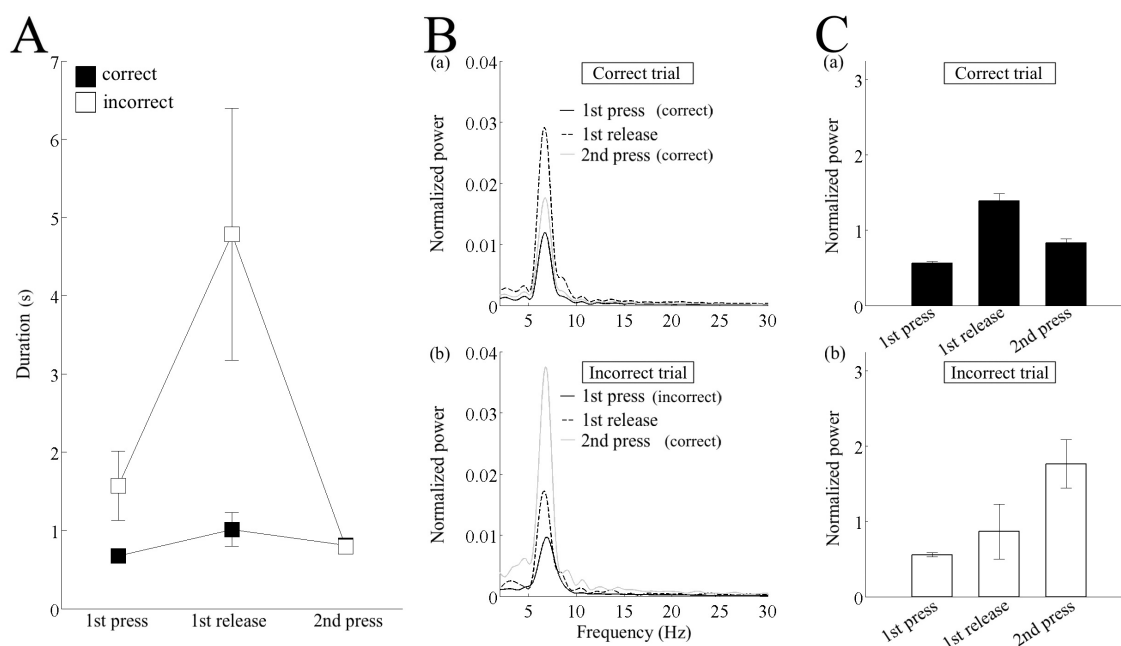
**Fig. 4. Increase in the relative and absolute theta power before the first lever press in the right- and left-forelimb trials.** (A) Dynamic power spectra before the first lever press with the right (a, c) or the left (b, d) forelimb in correct trials. The parameters used for the analysis were the same as in Fig. 3. The relative power (a, b) and the normalized absolute power (c, d) of each frequency were calculated as in Fig. 3. (B) Increase in the relative (a) and the normalized absolute (b) power of theta frequency band (6–9 Hz). The black and dotted lines indicate the data averaged over the right- and left-forelimb trials, respectively, and then over the rats. The shaded areas associated with the lines are standard error of the mean.



**Fig. 5. Increase in the relative and absolute theta power before the first lever press in the right and left robot-presentation trial.** (A) Dynamic power spectra before the first lever press in the right (a, c) or the left (b, d) presentation of the robot. The parameters used for the analysis were the same as in Fig. 4. The relative power (a, b) and the normalized absolute power (c, d) of each frequency were calculated as in Fig. 4. (B) Increase in the relative (a) and the normalized absolute (b) power of theta frequency band (6–9 Hz). The black and dotted lines indicate the data averaged over the each trial, and then over the rats. The shaded areas associated with the lines are standard error of the mean.

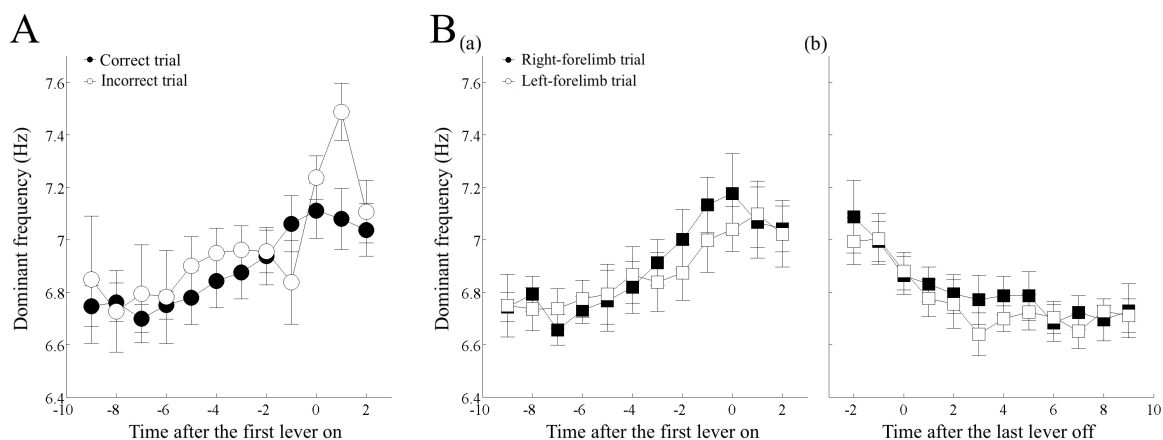


**Fig. 6. Decrease in the relative and absolute theta power after the termination of multiple lever pressing in the right- and left-forelimb trials.** (A) Dynamic power spectra after the last lever off with the right (a, c) or the left (b, d) forelimb in correct trials. The parameters used for the analysis were the same as in Fig. 3. The relative power divided by the total power of 1–30 Hz (a, b) and the absolute power subtracted and normalized by that at the time of lever off (c, d) were calculated for each frequency. The times before the last lever off are indicated as negative values. (B) Decrease in the relative (a) and the normalized absolute (b) power of theta frequency band (6–9 Hz). The black and dotted lines indicate the data averaged over the right- and left-forelimb trials in each rat, respectively, and then over the rats. The shaded areas associated with the lines are standard error of the mean.

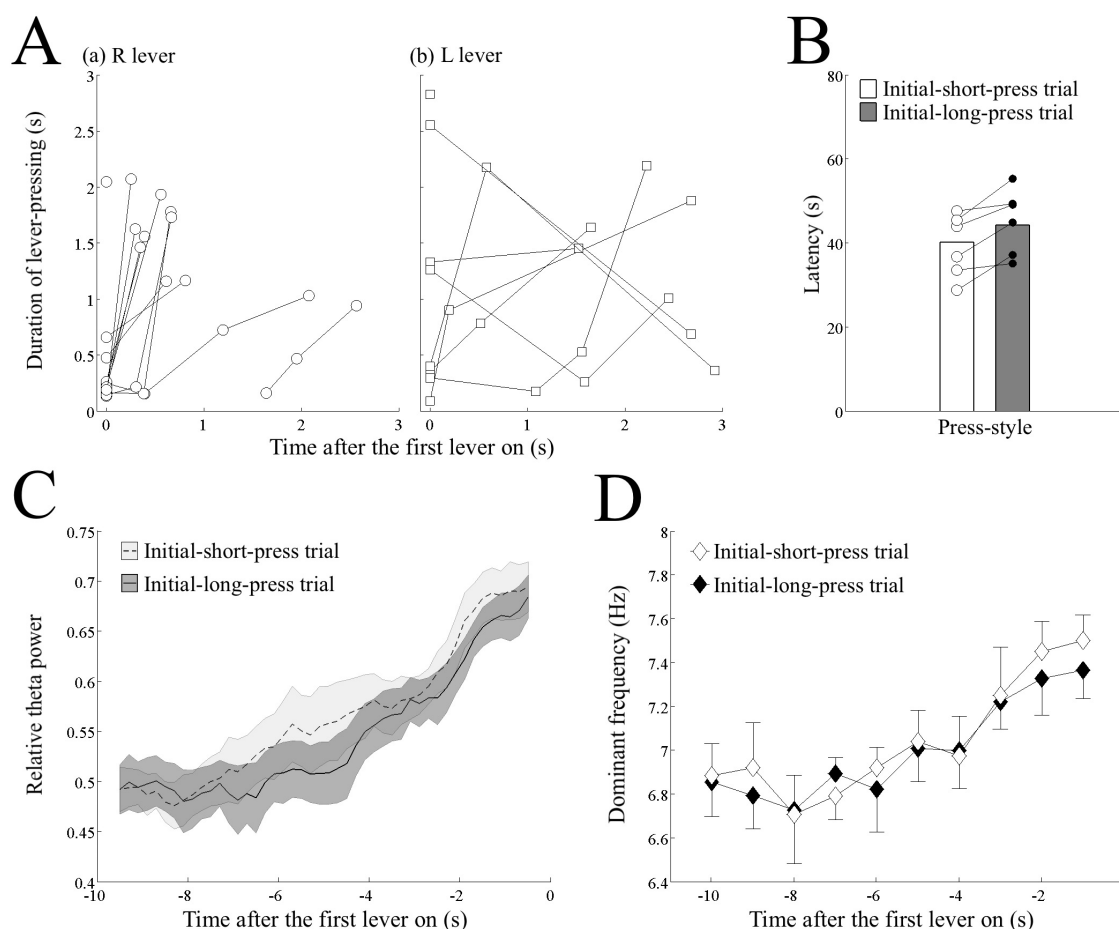


**Fig. 7. Differences between the correct and incorrect trials during the first and the second lever presses.** (A) Duration of the time between the first lever-on and the first lever-off (1st press), between the first lever-off and the second lever-on (1st release), and between the second lever-on and the second lever-off (2nd press). The data were averaged over the trial type in each rat and then overall the rats. (B) Absolute power spectral density normalized by the total power of the first press (see the methods for details) in the correct (a) and incorrect trials (b). The spectrum was averaged over the trial type in each rat, and then overall the rats. (C) Normalized power of theta frequency band (6–9 Hz) in the correct (a) and incorrect trials (b). The error-bars indicate the standard error of the mean.



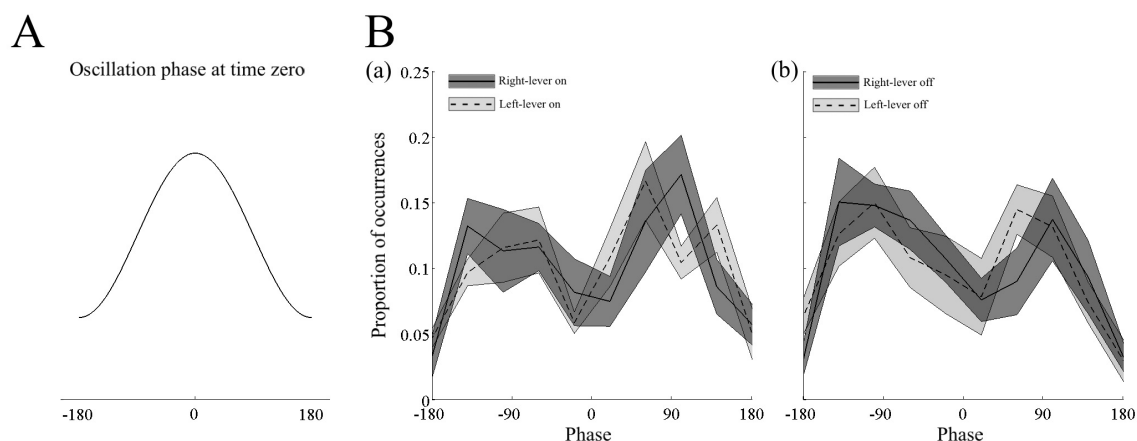


**Fig. 8. Change in the theta frequency before the first lever press and after the last lever release.** (A) Increase in the dominant frequency of theta oscillation before the first lever press in the correct and incorrect trials. The filled and open circles indicate the data averaged over the correct and incorrect trials in each rat, respectively, and then over the rats. (B) Increase in the dominant frequency of theta oscillation before the first lever press (a) and decrease after the last lever off (b) in the right and left forelimb trials. The filled and open squares indicate the data averaged over the right and left trials in each rat, respectively, and then over the rats. The error-bars indicate the standard error of the mean.

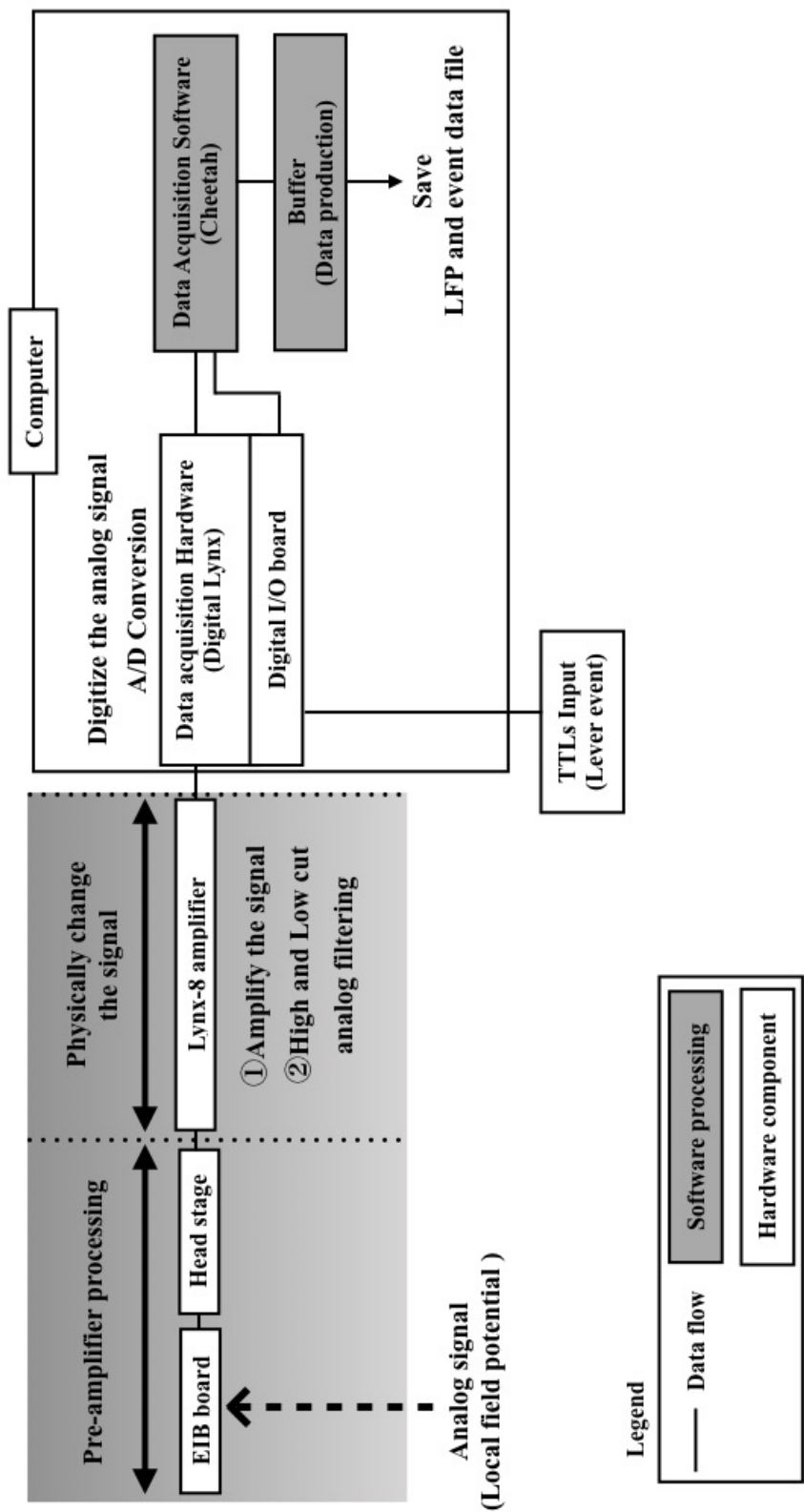


**Fig. 9. Differences between the initial-short- and initial-long-press trials before the first lever press in relative theta power and frequency.** (A) Duration of the lever-pressing in right (a) and left (b) trials during a session. Each open-circles and squares indicate the beginning of lever-on individually. The tied markers with a line represent a sequence of lever-on in a single trial. (B) Mean latency of inter-trial intervals. The open or filled circles indicate the average in each rat, and the bars indicate the average over the rats in each trial, respectively. The tied markers with a line correspond to the same rat. (C) Increase in the relative power of theta frequency band, which are integrated from 6–9 Hz. The black and dotted lines indicate the data averaged over the initial-short and initial-

long-press trials in each rat, respectively, and then over the rats. The shaded areas associated with the lines are standard error of the mean. (D) Increase in the dominant frequency of theta oscillation before the first lever press in initial-short and initial-long-press trials. The filled and open diamond indicate the data averaged over the initial-short and initial-long-press trials in each rat, respectively, and then over the rats. The error-bars indicate the standard error of the mean.



**Fig. 10. Instantaneous phase of first lever-on and last lever-off in the right- and left-forelimb trials.** (A) The scheme for the definition of phase (B) The phase, triggered in the first lever-on and -off, are averaged over right-forelimb and the left-forelimb trials in each rat, respectively, and then over the rats. The shaded areas indicate the standard error of the mean.



S1 Fig. Diagram of the environment for the data acquisition.

## Appendix

### 1 *Data acquisition system in the experiment*

The data recording was conducted under the cheetah acquisition system during the conditioning (S1 Fig.). The recorded local field potential via an electrode interface was pre-amplified from EIB board to the Head stage. In the first processing, the circuit input impedance is constructed highly so that the buffered preamplifier system converts a weak electrical signal into an output signal strong enough to be noise-tolerant. Then, the processed analog signal is amplified and applied high and low cut analog filter in the Lynx-8 amplifier in the second. After a series of this amplifier processing, the processed signal was digitized in the Digital Lynx so that the data fraction are processed.