Experimental Neurobiology
Vol. 18, pages 97~111, December 2009

Real-time One-dimensional Brain-Computer Interface (BCI) Using Prefrontal Cortex Neuronal Activities of Rats

Yi-Ran Lang, Hyunjoo Lee and Hyung-Cheul Shin*

Department of Physiology, College of Medicine, Hallym University, Chuncheon 200-702, Korea

ABSTRACT

The aim of this study is to verify the feasibility of control of one-dimensional (1-D) rotating machine using neural activities of Prefrontal cortex (PFC) in a BCI system. In this study, adult male Sprague-Dawley rats received bilateral implantation of recording micro-electrodes in PFC area. The spontaneous activities of a pair of PFC neurons of water-deprived rats were encoded and converted through a triple-step threshold comparator algorithm to three commands for one-dimensional movement control of a robotic wheel for accessing water. Averaged activities of two PFC neurons were quantized in every 200 ms to four ranges of activities around the mean firing rates (±0.5 SD) and were converted to four values. After comparison of the values of two chosen neuron units, direction and speed of rotation were decided. Rats were trained to complete one-dimensional control task to obtain water reward. The results indicated the percentage of stop event increased alone with more training. Different brain activity significantly influenced total water-drinking duration and non-water-drinking duration. Events generated from neuronal activity differed according to variant experimental sessions. Correlation between two signal units impacted controlling performance. Overall, the results of this study suggest that rats were able to manipulate the 1-D BCI system by differentially modulating PFC single neuron activities according to different circumstances.

Key words: brain-computer interface, prefrontal cortex, rat, one-dimensional, single neuron recording

INTRODUCTION

Brain Computer Interfaces (BCIs) enable motor disabled (Lucas et al., 2004) and healthy persons to operate electrical devices and computers directly with their brain activity. Recently, several groups began investigating the possibility of cortically controlled neural prostheses (Serruya et al., 2002; Taylor et al., 2002; Carmena et al., 2003; Shenoy et al., 2003; Kennedy et al., 2004; Musallam et al., 2004; Olson et al., 2005). Neuronal activities of premotor, primary motor and posterior parietal cortical areas of non-human primates were used to perform motor tasks (Johan et al., 2000) in BCI. Primates can learn to reach and grasp virtual objects by controlling a robot arm through a closed-loop brain-machine interface that uses mathe-

*To whom correspondence should be addressed.
TEL: 82-33-248-2585, FAX: 82-33-256-3426
e-mail: hcshin@hallym.ac.kr
Received October 17, 2009
Accepted for publication December 28, 2009

matical models to extract several motor parameters from the electrical activity of frontoparietal neuronal ensembles (Jose et al., 2003). Monkey primary motor neurons were decoded into a signal that is able to move a computer cursor to any new position in 2-dimensional space (Mijail et al., 2003). These known "read-out" research can transform the neuronal population signals into real-time prosthetic device movements. Unfortunately, such motor information cannot be obtained in patients with motor area disorders. The prefrontal cortex has long been associated with behavioural flexibility, working memory, planning, spatial navigation tasks, and goal-directed behaviour (Fuster, 1989). Despite the important functions of PFC, it has not been used in the study of BCI.

An understanding of how the brain represents movement facilitates the design of appropriate decoding algorithms, which forms the basis of main stream of BCI nowadays. Nonetheless, we hypothesized that it is not necessary to fully decoding neural signals. Simple models and algorithms might be sufficient for neural control of devices since the brain is a complex learning system which, with appropriate training, will learn to new and arbitrary tasks. This was one of our goals of developing a simple algorithm for rats to take advantage of BCI system using PFC area neuron activities.

MATERIALS AND METHODS

Implantation surgery and electrophysiology

Experiments were approved by the Hallym University Animal Care and Use Committee. Sprague-Dawley rats (230～280 g) were used in this study and they were succeeded in experimental animal center of Orient Bio co., Korea. The environment of breeding room was maintained at condition that temperature was 22±2°C and relative humidity was 55±10%. Artificial lighting maintained 12 hrs per day. Animals were housed 4 per cage with food and water was available ad libitum. Animals were anesthetized with ketamine (i.m., Yuhan, Korea, 100 mg/kg) and xylazine (Bayer Korea, Korea, 5 mg/kg). A relatively large craniotomy (2～3 mm diameter) was made bilaterally over the PFC. The PFC region (2.7 mm anterior to bregma, 0.5～1.0 mm lateral from midline, 1.8～2.0 mm ventral to the dorsal surface of the brain surface) of the left and right PFC was identified according to rat brain map (Paxinos and Watson, 1999), and one or two multi-wire recording electrodes of 8 channels (tungsten microwire, A-M systems, USA, 40 μm diameter, teflon-coated) were positioned with the tips of electrodes perpendicular to the cortex. Then it was lowered targeting the layer Ⅳ of PFC with hydraulic mover (Narishige, Japan). The 8-channels electrode (2×4) consisted of two bundles of four microwires. Each bundle was separated by approximately 1 mm and the interval between the adjacent microwires was about 50 μm. After verification of single neuron activity, the base of the electrode bundles was covered with a gelform and cemented altogether with dental resin to the pre- screwed anchors. After surgery, a recovery period at least one week was allowed before experimentation. One day prior to the experiment, the rats were deprived of water to enhance the rats' thirst. Each rat was moved into the experimental cage (Fig. 1A) and the rat was installed with the electrode head stages to check for the existence of neuronal units by using a data acquisition system (Plexon Inc., USA) for online multi-channel spike sorting and unit recording. Templates of the units were prepared and saved separately for each rat for later.

Algorithm for encoding neuronal activity for machine control and One-dimensional movement machine apparatus

Among simultaneously recorded many units, two units (N1 and N2) were selected and their activities were converted in every 200 ms to commands through a triple threshold comparator algorithm (Fig. 1B). There were 40 sec of pre-processing duration to calculate mean firing rate and SD in normal stage of rat. Activities of N1 and N2 neurons were counted and were converted to four activity ranges around the mean firing rates (M±0.5×standard deviation (SD)). This algorithm could adapt to any firing range and fluctuation and avoided difference of neural activity transition among rats and duration of trials. N1 was responsible for clockwise (CW) and N2 was responsible for counterclockwise (CCW) movement. The four firing ranges ($\geq$ (M+0.5×SD)+1, M～M+0.5×SD, M−0.5×SD～M−1, $\leq$(M−0.5×SD)−1) of N1 and N2 were normalized

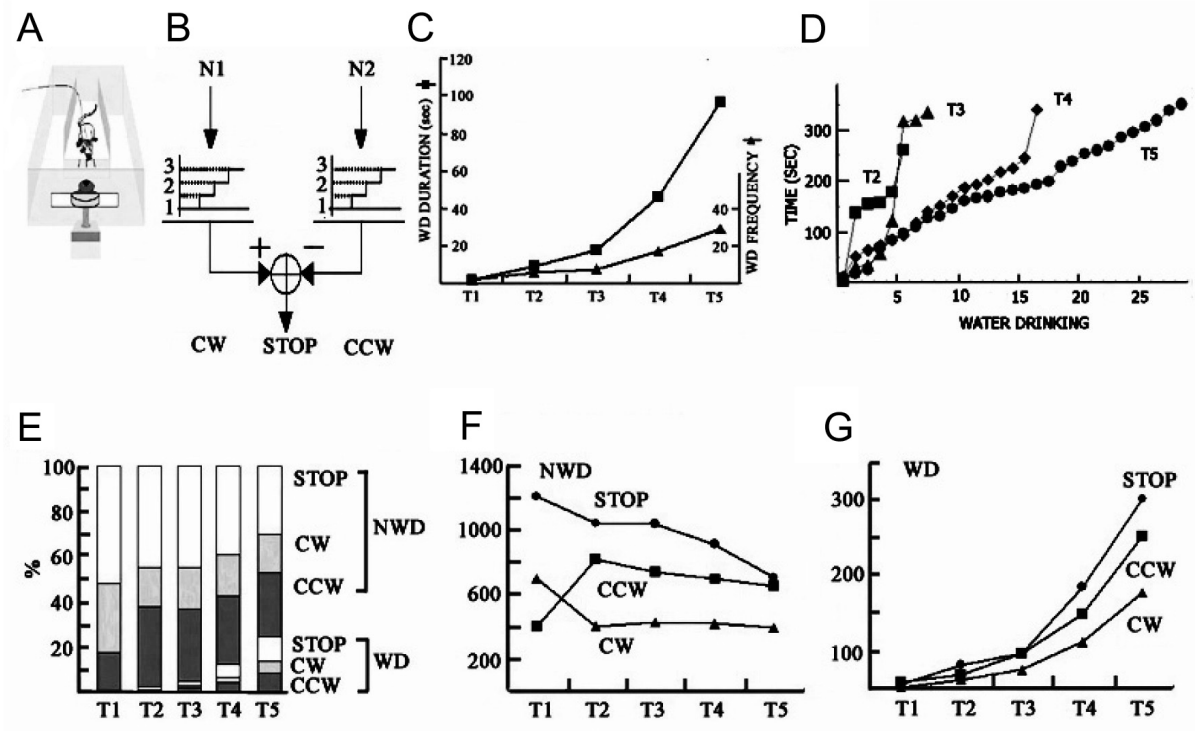

Fig. 1. (A) Setup of 1-D system. A computer interface rat could freely move in a chamber, facing a water containing disc in the same plane of chamber. (B) Triple-threshold algorithm for generating rotating command after comparing activities of two neurons. N1: neuron 1. N2: neuron 2. cw: clockwise, ccw: counterclockwise. (C) WD duration and WD frequency change along with trails. (D) Accumulation graph of WD in every trial. (E) Moving command distribution in percentage in NWD and WD duration. (F) Count of moving command changing along with trials in NWD duration. (G) Count of moving command changing along with trials in WD duration.

by assigning four command values of for CW (3, 2, 1, 0) and CCW (0, −1, −2, −3) rotation. Actual machine control commands were generated by addition of CW and CCW command values. The i80196 microprocessor received seven kinds of commands (3, 2, 1, 0, −1, −2, and −3 steps of rotation) from the control PC and executed it correspondingly. One-step of any direction of rotation turned the wheel exactly 14.5 degrees, two-step 21.5 degrees, and three-step 28.5 degrees, respectively. The direction of a rotation was determined by the polarity of the command. A positive direction resulted in CW rotation on rat side and negative direction in CCW rotation. When no turn (STOP) command was received, the stepper motor's magnetic force grabbed the axis of rotation very strongly, so it was impossible to rotate the wheel by external force. The black colored area of a disc (4 cm diameter) on a rotating wheel had water.

Four bits of digital event signals were fed from the control PC to the data acquisition system, and were recorded with neural signals. The bits were generated immediately before a specific command had commenced, and each bit indicated the exact timings of the CW-turn, CCW-turn, STOP and FLUSHING. 'FLUSHING' was needed to generate a compelled rotation of the wheel when the STOP command was continuously transmitted for 5 seconds. This was to disable any long-term water drinking, which could possibly result in the early termination of the session. FLUSHING rotated the wheel to the opposite direction of the previous rotation as far as the triple-steps degree (28.5°).

Experimental procedures

In the behavior chamber of the brain machine interface system, a rat was placed in front of a horizontally moving dish (diameter, 10.0 cm) with a circular dish (7 cm diameter) containing water in the black colored quadrant on top (Fig. 1A). The vertical position of the circular dish was the same as that of the floor of the cage. Drinking was made possible by positioning and keeping the water dish

in front of the rat. Three narrow flat steel platforms (4 cm long, 5 mm width) were placed at the floor edge in order to prevent grasping of the dish with forepaws. An experiment was begun only if a rat showed continuous (more than 40 sec. without turning its back to the chamber) water-seeking behavior 3 times over a 3 min period.

A session of wheel control for 6 min was followed by a 6 min of rest session while access to water was blocked by a paper wall. This sequence of wheel control and rest was repeated 3∼6 times until the rat showed no interest in water drinking. After the last trial, rats were allowed to eat food pellets (free food period, FF) while water access was blocked, and then were allowed to drink water freely for 6 min (free water period, FW) respectively. While eating food, during free water drinking, the actual wheel rotations were switched off but unit recordings and the command generations were recorded. A video camera recorded all the experimental sessions on the mechanical apparatus side in order to monitor the rats' behavior. Synchronization between the data acquisition system and video recordings was achieved using a light emitting diode that lit up at the start of the neuronal spikes recording. The timings of the beginning and the end of water drinking were manually marked by frame-by-frame reviews of the video recordings. These timings were fed into the computer in order to correlate the neural activities during either the water drinking (WD) periods or non-WD periods.

Data analysis

Statistical analysis was carried out using Graphpad Prism 4.0 (GraphPad Software, Inc., USA) and InStat 3.05 (GraphPad Software, Inc., USA). One way repeated measures of analysis of variance (ANOVA) was used to test statistical significance in difference in a single group of individual units among different behavioral context. All pair-wise multiple comparison procedures (Student-Newman-Keuls method) were also applied to isolate the group or groups that differ from the others. In every case, p values less than 0.05 were considered to indicate statistical significance.

Histological identification

For the histological analysis, animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains were removed and postfixed in the same fixative for 6 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Germany) into 30-μm coronal sections, and they were then collected into six-well plates containing PBS. Cresyl violet staining: To obtain the clear view of target area, the sections were mounted on gelatin coated microscopic slides. Cresyl violet (CV) acetate (Sigma, MO) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added in this solution. Before and after staining for 2 min at room temperature, the sections were washed twice in distilled water, dehydrated by placing successively for 2 hr in 50%, 70%, 80%, 90%, 95%, 100% ethanol bathes at room temperature, and finally mounted with Canada Balsam (Kato, Japan).

RESULTS

The experimental results were from 56 trials, 56 rest sessions, as well as FW, FF, and FAR sessions (total more than 600 min) in 16 rats. Every rat received 30 min to adapt to environment before experiment.

Fig. 1 and 2 show an example of machine control. Fourteen single units were simultaneously recorded and units 7b and 7d, which were isolated from a tungsten microwire, were used for machine control (Fig. 1A, B). Each machine control trial (T) lasting for six min. was followed by five min of resting period. This trial and rest sequence was repeated five times. We evaluated the efficiency of our BCI system using a paradigm of percentage of success in obtaining water reward as reported by Chapin et al. (1999). In this paper, overall wheel rotations in a trial comprised of repetitions of two periods, WD period and no-water drinking (NWD) period. Video analyses showed dramatic increases of WD frequency and total WD duration as trials

were repeated from T1 to T5 (Fig. 1C). Initially, total WD duration at T1 was less than 1% of 6 min, but that at T6 was over than 26%. This performance elevation was mainly due to by the increases of the WD frequency ((r)=0.9,950), since averaged WD durations (in sec, T1: 1, T2: 1.57±0.26, T3: 2.24±0.38, T4: 2.70±0.30, T5: 3.12±0.38) were not significantly different among five trials (p=0.0673, Kruskal-Wallis Test). WD events occurred intermittently at T2 and T3 but they were spread out for entire 6 min period of T5 (Fig. 1D).

Wheel rotations were controlled by CW, CCW, and Stop commands. Initially, STOP command was 52.17% of all commands generated during T1, but it was reduced to 41.24% during T5 (Fig. 1E). Although STOP commands during NWD period from T1 to T5 were gradually decreased (Fig. 1F), those during WD period were gradually increased as repeating trials (Fig. 1G). STOP commands during NWD period varied inversely to the WD frequencies from T1 to T5 ((r)=−0.9,804). During NWD periods from T1 to T5, changes of CW and CCW commands were shown in opposite direction to each other ((r)=−0.9,147). CW rotations were numerous at T1, but CCW rotations were dominant over CW from T2 to T5 (Fig. 1F). During WD periods, CW, CCW, and STOP commands were all increased (Fig. 1G) and changes of these commands were strongly related ((r)>0.99) to changes of both WD frequencies and total WD durations.

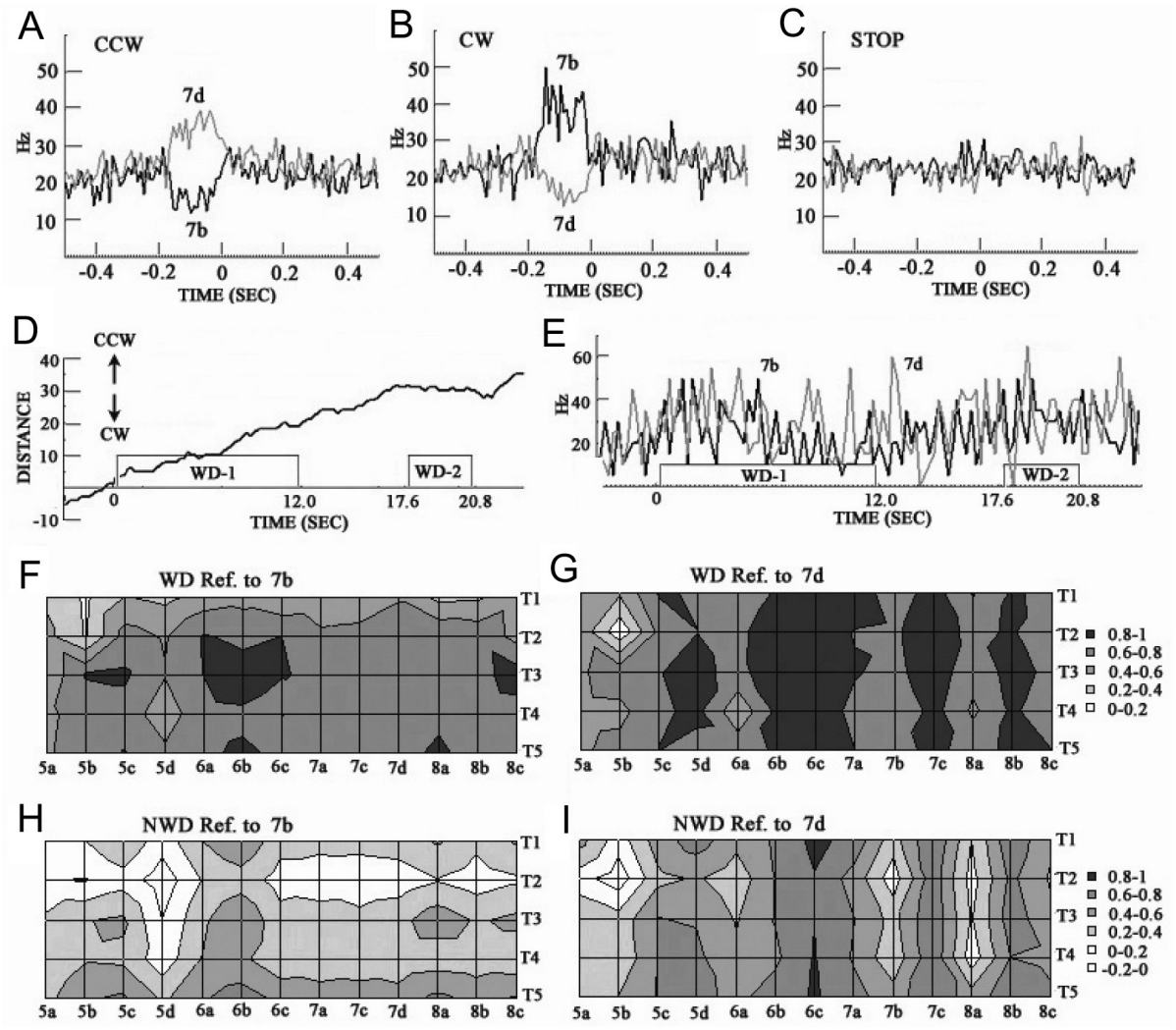

Fig. 2. (A) Opposite neuron activity change to produce CCW command. (B) Opposite neuron activity change to produce CW command. (C) Neuron activity generating STOP event. (D) The distance of disc within two WD durations. (E) Neuron activity happened in same duration with 2D. (F∼I) The correlation of other cells with reference to 7b and 7d from trial 1 to trial 5.

Neural activities of both 7b and 7d prior to command generation well reflected the operation of the triple-step threshold comparator algorithm (Fig. 1B). Neurons 7b and 7d were set to encode CW and CCW commands, respectively. Triple-step thresholds for unit 7b were set to 29, 27, 25 in Hz (CW threshold values: above 29: 3, 27～28: 2, 25～26: 1, below 25: 0) and those of 7d were to 27, 23, 19 in Hz (CCW threshold values: above 27: 3, 23～26: 2, 19～22: 1, below 19: 0). During 200 ms prior to either CW or CCW command generation, both neurons behaved antagonistically (Fig. 2A, B). For the T5 WD periods, neuron 7b showed elevated activity (35.95±1.72 Hz) to generate three steps of CW (i.e., threshold value 3 for CW), but neuron 7d exhibited suppressed activity (18.69±1.05 Hz, i.e., threshold value 0 for CCW) prior to CW commands. For the generation of CCW commands, neuron 7d showed heightened activity (33.23±1.16 Hz, i.e., threshold value 3 for CCW), but unit 7b exhibited lowered firing rates (17.91±1.04 Hz, i.e., threshold value 0 for CW). Stop commands were generated by not significantly different activity rates between both neurons (7b (23.68±0.72 Hz) vs. 7d (21.34±0.57 Hz): $p>0.05$, Fig. 2C). However, corresponding threshold values for 7b and 7d were CW 0 and CCW 1, respectively. This appeared to contradict to the triple-threshold comparator algorithm to generate Stop commands. The fact that 7d's activities were not significantly different ($p>0.05$) for the generations of CCW 1 and CCW 0 suggested that Stop commands were possible mainly when both neurons' activities were at threshold value 0. Neuron 7b showed significantly different firing rates for each of three commands (CW vs. CCW, $p<0.001$, CCW vs. Stop, $p<0.01$, CW vs. Stop, $p<0.001$) and threshold values segregated into two levels and fluctuated mainly between CW 3 and CW 0. Neuron 7d showed distinctively different activities between CW vs. CCW ($p<0.001$) and between CCW vs. Stop ($p<0.001$) and threshold values changed among CCW 3, CCW 1 and/or 0. Thus, CCWs had three-step movements, while CWs had either three or two-step movements. This ensured the dominant generation of CCW over CW. Similar activity ranges of neuron 7d also endowed the flexibility to generate either CW 2 or Stop commands. Although for background periods of 301 to 500 ms prior to and 500 ms post to CW command generation neurons 7b (24.35±0.45 Hz) and 7d (24.21±0.42 Hz) showed similar firing rates, 7b exhibited significantly lower activities than 7d (7b, 22.20±0.40 Hz; 7d, 24.39±0.36 Hz, $p<0.001$) for corresponding background periods to CCW command generation. Thus, 7b more strictly obeyed the triple-step threshold algorithm than 7d did during WD periods of T5, suggesting a flexible role by 7d to generate more Stops to maintain water access and more CW rotation to counteract the CCW movement. During this WD period of T5, overall, Stop commands were the most frequent and CW commands were the least (in %, Stop, 43.33, CCW, 34.83, CW, 21.84).

During NWD periods of T5, activities of neuron 7b and 7d also showed similar trends for command generations as in WD periods. However, in contrast to WD periods, both neurons exhibited distinctively different firing rates for each of three commands (in Hz, Neuron 7b: CW, 35.74±1.45, CCW, 18.47±0.75, Stop, 23.93±0.53, CW vs. CCW, $p<0.001$, CCW vs. Stop, $p<0.001$, CW vs. Stop, $p<0.001$; Neuron 7d: CW, 18.58±0.82, CCW, 33.20±1.02, Stop, 22.38±0.43, CW vs. CCW, $p<0.001$, CCW vs. Stop, $p<0.001$, CW vs. Stop, $p<0.05$). Both neurons also showed significantly different background firing rates for 301 to 500 ms prior to and 500 ms post to each of three commands generation (in Hz, Neuron 7b: CW, 25.54±0.30, CCW, 23.53±0.21, Stop, 23.61±0.20; Neuron 7d: CW, 24.47±0.31, CCW, 25.78±0.22, Stop, 24.87±0.23; 7b vs. 7d: CW, $p<0.05$, CCW, $p<0.001$, Stop, $p<0.01$). Thus, during NWD periods, both neurons followed strictly to the triple-step threshold algorithm than during WD periods of T5. During this NWD period of T5, overall, Stop commands were the most frequent and CW commands were the least (in %, Stop, 40.23, CCW, 37.19, CW, 22.58).

During 6 min of T5, there were 29 WD and 30 NWD periods. Fig. 2D shows wheel movements by two WD and three NWD periods. In this example, WD-1 consisted of several repetitions of two different modes of water drinking, i.e., water drinking while issuing CCW wheel rotations (WD-CCWs) and water drinking during fine control (WD-FCs) of wheel rotations while generating combinations of three commands. During WD-CCWs, water-contain-

ing region of the rotating wheel moved toward rat at the beginning of the WD-1 period and it moved away from the rat at the later period of the WD-1. During WD-FCs, the wheel movements were maintained around a fixed position because of many Stop commands and antagonistic actions between CW and CCW commands. In contrast to WD-1 period, WD-2 period was consisted of many WD-FCs. In fact, WD-1 period was consisted of 23 CCWs, 7 CWs and 30 STOPs, while WD-2 period was made of 4 CCWs, 4 CWs and 13 STOPs. More numerous CCWs than CWs commands during WD-1 period was guaranteed by the fact that mean firing rate of 7d (29.79±1.92 Hz, i.e., threshold value 3 for CCW) was significantly ($p=0.0186$) higher than that of 7b (23.51±1.79 Hz, i.e., threshold value 2 for CW, Fig. 2E). During WD-2 period, mean activity of 7b (31.47±2.53 Hz, i.e., threshold value 3 for CW) was similar to that of 7d (30.59±3.45 Hz, i.e., threshold value 3 for CCW), such that they ensured more frequent generations of Stop (i.e., CCW 3−CW 3=0) command.

As shown above, two units (7b and 7d) were directly responsible for command generations. Throughout T1-T5, units 7b and 7d showed strong positive correlations ($(r)>0.6$) between them during WD periods, but they did not have significant relations during NWD periods. Simultaneously recorded other 12 neurons near to 7b and 7d were also involved in either directly or indirectly to the command generations. They were differentially related to activity changes of either 7b or 7d during WD and NWD periods (Fig. 2F∼I, Table 1). In general, unit 7d (Fig. 2G, I) showed higher positive correlations to other neurons than unit 7b did (Fig. 2F, H), regardless of WD or NWD periods. Both neurons exhibited stronger correlations with other neurons during WD period (Fig. 2F, G) than during NWD period (Fig. 2H, I). Thus, these suggest that highly concerted influences from other neurons might be required for the flexible role of unit 7b to generate appropriate commands for water access during WD periods. Eight units (5c, 5d, 6b, 6c, 7a, 7c, 8b, and 8c) showed strong correlations ($(r)>$

Table 1. Correlations of controlling signal 7b and 7d with other signals

Ref 7d		5a	5b	5c	5d	6a	6b	6c	7a	7b	7c	8a	8b	8c
WD	T5	0.57	0.67*	0.80*	0.74*	0.74*	0.82*	0.89*	0.80*	0.75*	0.84*	0.74*	0.83*	0.80*
	T4	0.53	0.54	0.77*	0.84*	0.45	0.84*	0.92*	0.75*	0.61*	0.86*	0.56	0.83*	0.68*
	T3	0.57	0.73*	0.80*	0.83*	0.76*	0.89*	0.93*	0.82*	0.74*	0.91*	0.67*	0.88*	0.74*
	T2	0.53	0.05	0.65*	0.80*	0.75*	0.94*	0.90*	0.79*	0.77*	0.89*	0.75*	0.82*	0.60*
	T1	0.57	0.43	0.82*	0.78*	0.66*	0.82*	0.87*	0.90*	0.70*	0.88*	0.58	0.82*	0.76*
Ref 7b		5a	5b	5c	5d	6a	6b	6c	7a	7b	7d	8a	8b	8c
WD	T5	0.57	0.71*	0.81*	0.65*	0.78*	0.82*	0.77*	0.76*	0.76*	0.75*	0.83*	0.74*	0.76*
	T4	0.55	0.72*	0.71*	0.48	0.76*	0.77*	0.60*	0.64*	0.60*	0.61*	0.77*	0.68*	0.70*
	T3	0.43	0.81*	0.83*	0.61*	0.80*	0.89*	0.82*	0.71*	0.76*	0.74*	0.72*	0.72*	0.90*
	T2	0.45	0.17	0.68*	0.58	0.81*	0.75*	0.80*	0.64*	0.67*	0.77*	0.78*	0.74*	0.53
	T1	0.37	0.16	0.41	0.35	0.47	0.52	0.42	0.44	0.48	0.57	0.34	0.37	0.52
Ref 7d		5a	5b	5c	5d	6a	6b	6c	7a	7b	7c	8a	8b	8c
NWD	T5	0.27	0.40	0.61*	0.65*	0.56	0.68*	0.82*	0.66*	0.49	0.77*	0.50	0.72*	0.63*
	T4	0.23	0.22	0.60*	0.64*	0.44	0.57	0.81*	0.58	0.26	0.76*	0.09	0.60*	0.56
	T3	0.39	0.27	0.60*	0.59	0.38	0.61*	0.80*	0.61*	0.33	0.75*	0.17	0.71*	0.45
	T2	0.07	−0.12	0.34	0.42	0.27	0.60*	0.79*	0.52	0.09	0.72*	0.12	0.65*	0.38
	T1	0.49	0.09	0.50	0.71*	0.46	0.62*	0.85*	0.74*	0.29	0.80*	0.20	0.75*	0.44
Ref 7b		5a	5b	5c	5d	6a	6b	6c	7a	7b	7d	8a	8b	8c
NWD	T5	0.34	0.57	0.63*	0.36	0.58	0.70*	0.55	0.50	0.54	0.49	0.65*	0.54	0.62*
	T4	0.20	0.38	0.36	0.08	0.41	0.44	0.25	0.27	0.25	0.26	0.28	0.28	0.26
	T3	0.24	0.39	0.43	0.00	0.36	0.56	0.28	0.33	0.26	0.33	0.52	0.37	0.45
	T2	0.01	−0.01	0.12	−0.12	0.21	0.32	0.12	0.07	0.08	0.09	0.19	0.09	0.17
	T1	0.20	0.19	0.37	0.09	0.40	0.55	0.28	0.28	0.26	0.29	0.42	0.26	0.42

*$p<0.05$.

Table 2. Neuron activities 200 ms prior to STOP, CCW, and CW commands were correlated to Ref 7b and 7d in WD and NWD durations

WD		5a	5b	5c	5d	6a	6b	6c	7a	7b	7c	7d	8a	8b	8c
Stop	Ref 7b	−0.11	0.29	−0.14	−0.12	0.10	0.30	−0.27	0.20		−0.30	−0.11	−0.07	0.02	−0.06
	Ref 7d	−0.44	−0.22	0.23	−0.27	−0.07	−0.12	−0.44	−0.48	−0.11	−0.24		0.06	−0.14	0.16
CCW	Ref 7b	0.22	0.19	0.16	0.01	0.18	−0.28	−0.10	−0.24		0	−0.54*	0.24	−0.41	−0.24
	Ref 7d	−0.14	0.05	0.25	0.45	−0.07	0.31	0.30	0	−0.54*	0.11		−0.01	0.34	0.49
CCW	Ref 7b	0.31	0.27	0.32	0.22	0.09	0.33	−0.01	0.15		−0.13	−0.61*	0.30	0.32	0.22
	Ref 7d	−0.30	−0.12	−0.31	−0.28	−0.39	−0.01	0.28	−0.24	−0.61*	0.14		−0.17	−0.04	−0.36
NWD		5a	5b	5c	5d	6a	6b	6c	7a	7b	7c	7d	8a	8b	8c
Stop	Ref 7b	−0.32	0.12	−0.05	0.30	0.15	0.36	0.20	−0.18		0.05	−0.04	0.02	0.395	−0.21
	Ref 7d	−0.21	−0.05	0.06	−0.24	−0.16	0.41	0.07	−0.25	−0.04	−0.02		0.23	−0.250	0.12
CCW	Ref 7b	−0.21	0.19	−0.29	−0.33	0.12	−0.33	−0.64*	0.21		−0.51	−0.88*	−0.09	−0.310	−0.05
	Ref 7d	0.04	−0.22	0.36	0.39	−0.09	0.32	0.64*	0.11	−0.88*	0.25		0.14	0.430	−0.06
CW	Ref 7b	−0.07	0.33	−0.13	−0.07	0.20	0.21	−0.08	0.29		−0.10	−0.62*	0.40	−0.070	0.29
	Ref 7d	0.28	−0.25	0.16	0.12	−0.11	−0.06	0.34	−0.43	−0.62*	−0.02		−0.14	0.250	0.05

*$p < 0.05$.

0.6) to unit 7d during WD periods throughout five trials, but only three units (6c, 7c, and 8b) exhibited strong correlations during NWD (Table 1). None of 12 units showed any correlations to unit 7b during WD period of T1, but seven units (5c, 6a, 6b, 6c, 7a, 7c, 8a, and 8b) exhibited strong relations during WD period of T2. During WD period of T3 and T5, all units except unit 5a had strong relations to 7b. During T4, all units, except 5a and 5d, exhibited strong relations to unit 7b. Thus, from T2 to T5, six units (5c, 6b, 6c, 7a. 7c, and 8b) showed strong correlations to both 7b and 7d neurons during WD periods. However, unit 5a was not correlated to both neurons regardless of WD or NWD period. Unit 5d and 8c had more preferential correlations to unit 7d than to unit 7b during WD periods of T1-T5, but units 5b, 6a, and 8a showed preference to unit 7b than unit 7d. Thus, units 5d and 8c might preferentially modulate unit 7d's activity to generate threshold values of either CCW 0 or CCW 1. Units 5b, 6a, and 8a might be preferentially involved in flexible control of unit 7b's activity to generate threshold values of either CW 0 or CW 3. However, these preferential influences to 7b and 7d appeared to be indirect since cross-correlation analyses did not show any immediate and presumable synaptic modulations. During NWD periods throughout T1～T5, unit 7b was not related to most of simultaneously recorded units, except three units (5c, 6b, and 8a) at T5. Three units (5a, 5b, and 6a) were related to neither 7b nor 7d units during NWD periods throughout T1～T5.

Other simultaneously recorded neurons' activities at T5 were also analyzed to see whether they had any relations to either unit 7b or 7d during 200 ms prior to each command generation (Table 2). During overall WD period of T5, both 7b and 7d did not show any significant correlations to other simultaneously recorded neurons. However, 7b and 7d exhibited negative relation ((r)=−0.61) to each other prior to CW command generation and mild relation (but significant, (r)=−0.54) between them prior to CCW command generation during WD periods of T5. During NWD periods of T5, much stronger negative correlations between two units were found prior to CCW generation ((r)=−0.88) than prior to CW output ((r)=−0.62). Unit 6c exhibited strong positive correlation to unit 7d and naturally negative relation to unit 7b during NWD period. These correlation analyses coincided with CCW dominance (i.e. threshold value 3 for CCW) during NWD period and the importance of CW movements (i.e. threshold value 3 for CW) during WD period. Units 7b and 7d did not showed any significant correlations between them prior to generating Stop commands during both WD ((r)=−0.1076) and NWD ((r)=−0.0414) periods of T5. This was natural since threshold value for CW 0 was generated concertedly by two different mean firings of 7b during both WD and NWD periods and threshold values for CW 0 and CW 1 were determined by not significantly different 7d's mean

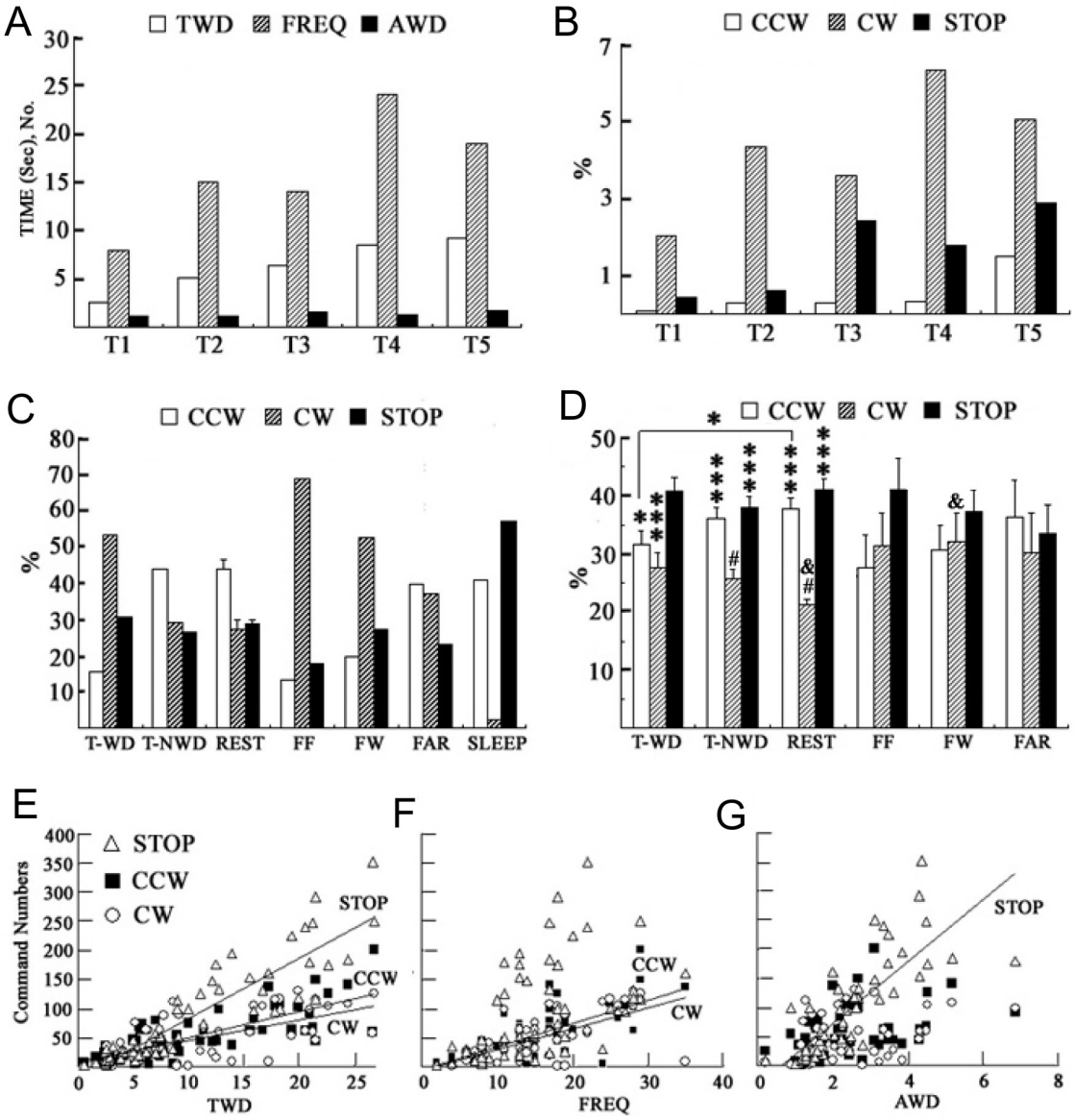

Fig. 3. (A) Histogram of total WD, Frequency, and AWD in five trials. (B) Histogram of total WD, Frequency, and AWD in percentage. (C) Histogram of CCW, CW, and STOP in different sessions. (D) Statistic comparison of CCW, CW, and STOP in different sessions. (E～G) Correlations of CW, CCW, and STOP with TWD, Frequency, and AWD.

firing rates responsible for the two commands as stated earlier during WD period.

Fig. 5F illustrates the neuronal activity in pre- and post- WD duration. Bin size is 1 sec. Neuron activity significantly increased in WD than pre-WD ($p<0.01$). And post-WD neuron activity gradually decreased in 10 sec. after WD. In an experiment, trial-dependent increase of TWD (%) was strongly ((r)=0.9053) correlated to the change of WD frequency (Fig. 3A). This trial-dependent WD frequency augmentation was mainly related to the changes of CW ((r)=0.9920) movement and AWD duration was highly related to the occurrence of Stop ((r)=0.9419) command (Fig. 3B). If water dish control using the BCI system to quench thirstiness was intentional, proportions of three commands generation might be different among various behavioral states. During 6 min of sleep period, where rats did not have any intention to drink water and thereby no intention to control the BCI system, CW command was few while Stop command was numerous (Fig. 3C). During WD periods of T5, CW was dominant whereas CCW was the least. However, during NWD periods of T5 (T-NWD), CW

and CCW were observed in similar proportions. Command proportions during this NWD periods were quite similar to those when water dish control was allowed but accessing water was blocked by placing water dish at a distance away from the rat (FAR in Fig. 3C). This is natural since during T-NWD and FAR period the rat had intention to control the BCI system but it experienced difficulty of accessing the water dish. During 6 repetition of resting (REST) periods in between 5 trial periods the rat had to wait for 6 min to start new WD trial since the rat's chamber was blocked by a panel and thereby it had to gave up the intention of controlling the BCI system. At this period CCW command was most frequently observed. Proportions of three commands generated when the rat was allowed to access water freely (FW) by fixing the water dish in front of the rat were similar to those of T-WD. However, CW and CCW frequencies were different between two behavioral states. Proportions of three commands generated when the rat was allowed to access food freely (FF) by placing food pellets in the rat chamber were similar to those of T-WD. However, CCW and Stop frequencies were different between two behavioral states. Overall, generally, proportions of command generations during T-WD, FF, and FW periods showed numerous occurrence of CW command, whereas those during T-NWD, REST, FAR, and SLEEP periods exhibited frequent CCW command. Fig. 3D shows averaged proportions of three command generations from 16 rats during various behavioral states (T-WD: n=59, T-NWD: n=59, REST: n=102, FF: n=14, FW: n=14, FAR: n=4). During T-WD, Stop command was significantly numerous than either CW or CCW commands. However, during both T-NWD and REST periods CW command was significantly fewer than either CCW or Stop commands. It was also noticed that CCW occurrence during REST was significantly more frequent that that during T-WD. Averaged proportions of three command generations were similar during FF, FW, and FAR periods. During REST, CW occurrence was significantly fewer than

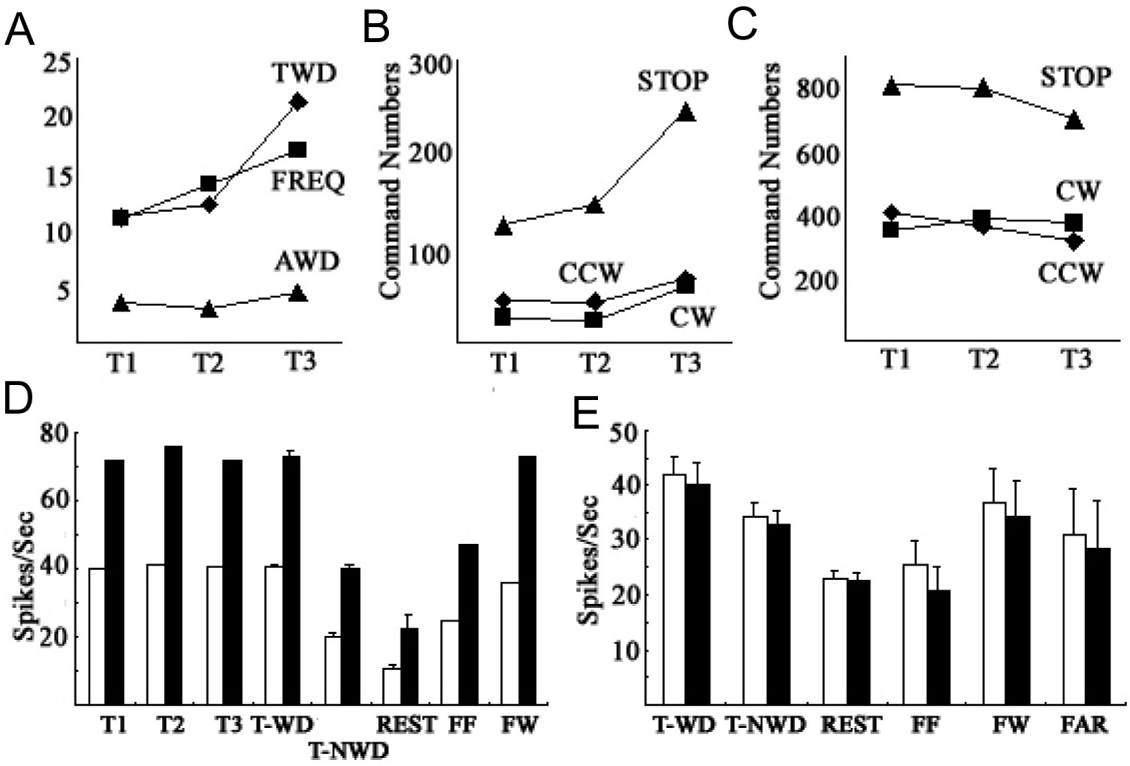

Fig. 4. (A) TWD, Frequency, and AWD change in the first three trials. (B) CCW, CW, and STOP change in the first three trials in WD duration. (C) CCW, CW, and STOP change in the first three trials in NWD duration. (D) Activities of two controlling neurons in different sessions. (E) Statistic analysis of their activities in different sessions.

that during either T-NWD or FW period. Relationships between BCI efficiency (TWD, FREQ, and AWD) and three commands generated during T-WDs from 56 trials in 16 rats were analyzed (Fig. 3E～G). TWD changes from trial to trial showed linear correlations to all three commands (CCW: (r)=0.7696, CW: (r)=0.6596), but with strongest relation to the Stop command ((r)=0.8980). WD frequency was linearly related to both CCW ((r)= 0.6982) and CW ((r)=0.6732) commands. AWD duration was related to the Stop command ((r)= 0.7922).

In an experiment, TWD (%) and WD frequency was increased from T1 to T3 (Fig. 4A). During T-WD periods frequencies of Stop ((r)=0.9,970) movements were related to TWD changes (Fig. 4B). At T3 all three commands were increased. During T-NWD periods both Stop and CCW commands were decreased from T1 to T3 (Fig. 4C). In this experiment Neuron 1d and 11c were set to encode CCW and CW commands, respectively (Fig. 3D). Triple-step thresholds for unit 1d were set to

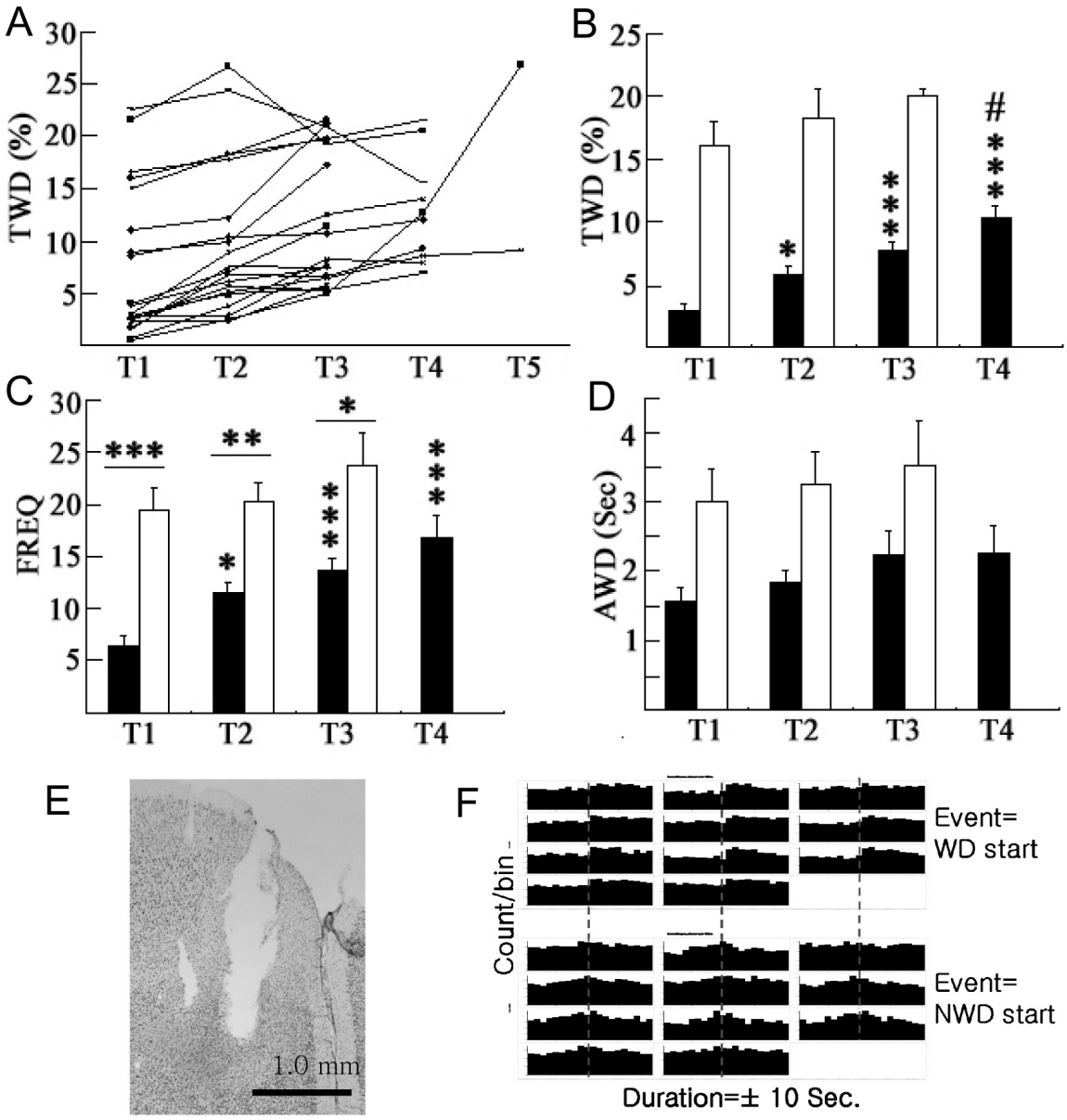

Fig. 5. (A) Percentage of TWD in all experiments. (B～D) Change of TWD, Freq., and AWD of all experiments in the first four trials, black: 13 of 20 rats, white: 7 of 20 rats. (E) Histology of implantation site on PFC. (Cresyl violet stain). (F) Perievent histogram of 11 signals. The dotted line shows the initial of WD (above) and NWD (below).

30, 20, 10 in Hz (CCW threshold values: above 30: 3, 20～29: 2, 10～19: 1, below 19: 0) and those of 11c were to 60, 40, 20 in Hz (CW threshold values: above 40: 3, 40～39: 2, 20～39: 1, below 20: 0, Fig. 4D). Firing rates of both 1d and 11c were above each neuron's threshold level 3 during T-WD periods from T1 to T3. During T-NWD periods activities of both 1d and 11c were at ranges to generate each neuron's threshold value either 2 or 1. During resting periods, both neurons' firings were at levels for generating threshold values either 1 or 0. Average activity of 1d during rest was significantly lower from that during T-NWD ($p=0.0024$) and that during T-WD ($p=0.0238$). Average activity of 11c during rest was also significantly lower from that during T-NWD ($p=0.0238$) and that during T-WD ($p<0.0001$). Activities of 1d and 11c during FW period were similar to those during T-WD period. Firings of both neurons during FF were similar to those during T-NWD period. Averaged activities of N1s and N2s from 16 rats during resting period were significantly lower than those during either T-WD or T-NWD (Fig. 4E).

In Fig. 5, trial-dependent BCI efficiency changes obtained from 20 rats were illustrated in terms of % TWD duration, frequency of WD and AWD duration per trial. TWD (%) at T1 were variable from 0.56% to 21.54% of 6 min (Fig. 5A). Thirteen of 20 rats showed trial-dependent gradual increase of BCI efficiency in terms of TWD and wd frequency (Fig. 5B, C, black). Most of them showed less than 5% of TWD at T1. Seven of 20 rats not showing trial-dependent BCI efficiency increase tended to exhibit higher TWD (8.96～22.67%) even at T1 (Fig. 5B, C, white). Averaged WD durations per trial were not altered as trials were repeated. Thus BCI efficiency augmentation occurred when initial efficiency was quite low and it is mainly done with the increment of WD frequency. WD amount was measured after each trial and averaged WD amount per sec was 0.049±0.010 ml.

DISCUSSION

Teuber (1972) hypothesized that the PFC neurons play a vital role in processing signals from sensory to motor. Here, we demonstrated that PFC (Fig. 5E) neuron could function as an adaptive goal-encoding region to fulfill an animal's requirements when spontaneous signals were transformed in real-time as commands for controlling a machine through a simple brain computer interface system. In particular, this study represented following: (1) a simple algorithm was developed for rats to take advantage of BCI system using a PFC single neuron activities. (2) PFC neurons provided a feasible adaptive choice for animals to manipulate machine with BCI efficiency increase by learning from several training sessions. (3) Intentional control of our BCI system was evidenced by differential changes of neuronal activities and consequent generations of three commands during various behavioral states, such as resting, free food, free water, sleeping and FAR periods.

A closed loop of information stream was formed under elaborate design of BCI system: a thirsty rat with intention, triple-threshold algorithm (Fig. 1) to convert neural signal to machine control commands and command executing system. This circle was completed when rat is starting to notice and understand the function of the reward containing system. To investigate whether rats could be trained (encode) to comprehend the relation between themselves and moving disc, they were required to finish task of controlling drinking water in rotating 1-D system. Trial-dependent BCI efficiency increase was quite prominent especially when initial efficiency at trial 1 was very low (Fig. 5). However, when initial efficiency was high above 10% of 6 min, rats showed less intention to control the BCI system after 3 trials. Intentional differences were also expressed in the proportions of generated three commands. Sleep period had no intention to drink water and thereby no intention to control the BCI system. The proportions of three commands generated during this sleep period were quite different from those during WD trial periods (Fig. 3C). During the 6 min of resting periods in between the trial periods, the rat had to wait for next trial with intention of WD. Although it is a short interval, the rat also had to wait for the next T-WD period during the T-NWD periods in between the T-WD periods (Fig. 5F). This similarity was well reflected in the proportions of three commands between the T-NWD and resting periods (Fig. 3C, D). For trained rats, the rotating disc serves as a

visual cue. The NWD periods are delay duration in a task. This might explain the persistence of neuron activity which results in different proportions of commands from resting and FAR sessions (Fuster, 1973). During the FAR period the rat could watch the water dish movement with the intention to have water, but it could not access the water for 6 min since the water dish was pulled away from the rat. Command generations during FW, which was tested after several WD trials, were basically similar to those in T-WD even though there was no actual control of water dish movement during FW period. Command generations during FF shown in Fig. 3C appeared similar to those during FW, but CW was further increased while CCW and stop commands were decreased. The similarity may manifest the common intention to fulfill its appetite, but the-difference may be related to the absence of intentional control of the water dish, since food pellets were given freely in the chamber, not on the controllable water dish. Proportions of three command generations during FF, FW, FAR periods were quite variable among 16 rats and they were not significantly different among each other (Fig. 3D), suggesting the necessity of strict regulation of command generation was commonly absent compared to those during WD trials.

In this study, the maximum BCI efficiency was lower than 30% and the efficiency was increased by the elevation of WD frequency. If we suppose that the system was rotated in one direction with a specified angle for a trial without stop command, the rat could access the water dish for 86°. In this case, the efficiency is 23.9% and one cycle of rotation takes 3.35 sec for 3 steps (21.5°) and 4.97 sec for 1 step (14.5°) angular movements. Thus, estimated averaged WD duration ranges from 0.80 ~1.19 sec and WD frequencies are 72.5~107.5 times for 6 min of a trial period. In our study, overall averaged WD duration was 2.37±0.17 sec for whole trials (n=56) and the averaged WD frequency was 15.09±0.99 times for a trial period (highest WD frequency was 35). These indicate that neither actual BCI controls were done by simple rotation in one direction (Fig. 3F) nor water drinking was done by chance during the wheel rotation. The occurrence of stop command during fine control period aided to increase the averaged WD duration (Fig. 3G), but it mostly decreased the WD frequency during non-WD period of a trial. One to one counter actions of two directional commands were good for fine control period and for the WD frequency increase (Fig. 3F). However, dominant command generation for one direction during non-WD period acted to speed up the next access of water containing region (Fig. 3D). The least performance of the current BCI system could be possible by fixing or moving the water dish at the place non-accessible by the rat. This region covers 72.1% of the wheel rotation which could move 360 o. On the other hand, the best performance of our BCI system could be possible by moving the water containing region, while issuing 1 to 1 ratio of both CW and CCW commands and many stop commands (Fig. 3G), right in front of the rat, which covered 23.9% of the wheel rotation. This sort of best performance was shown as WD-FC during WD-2 period in Fig. 2D. Compared with decoding algorithms, encoding algorithm requires less signals (minimum 2 signals), to reach high efficiency which in return could performance more functions using similar number of neurons. Accordingly, this algorithm causes less damage of using massive implantation to brain tissue by immune reaction in other researches, although decoding accuracy is heightened by massive electrodes.

Due to the remarkable cortical plasticity of the brain, signals from implanted electrode can, after adaptation, be handled by the electrodes and computers (Levine et al., 2000). Activities of N1 and N2 neurons well followed the triple threshold comparator algorithm to generate corresponding commands (Fig. 2A~C and Fig. 4D). Because PFC neurons perform various functions like prediction, working memory and discrimination, the mean, maximum, minimum frequency of PFC neuron in a rat or among different rats could not be consistent. The average spontaneous firing rate of population of neurons was 5.9±1.6 (S.D.) spikes/s. Although each neuron's absolute activity level was important for the generation of a normalized threshold value, the differences (3, 2, 1, 0, −1, −2, −3) of two normalized threshold values (3, 2, 1 and 0) encoded from two neurons' activities were the actual determinant for one of three commands (CW: 3, 2, 1; CCW: −3, −2, −1; Stop: 0). This algorithm,

thus, made two neurons to fire in negative correlation, even though the two neurons were recorded from a single wire, for the generation of CW and CCW commands and in no correlation for Stop command during the pre-command 200 ms period (Fig. 2A, B, Table 2). The effectiveness of this triple threshold comparator algorithm was mostly restricted to the designated N1 and N2 neurons although there were many neurons in the vicinity of the two neurons (Table 2). It fully reflects that PFC is not construed simply as cluster of neurons but as a network (Fuster, 1989).

Because of the nature of BCI algorithm, trial-dependent increase of three commands and concomitant BCI efficiency elevation were possible even when two neurons' activities among WD trials were not much different (Fig. 4D). Trial-dependent BCI efficiency increase was mainly due to the ascension of WD frequency (Fig. 5C) since averaged WD duration was not significantly changed as repeating trials (Fig. 5D). WD frequency changes were significantly correlated to occurrences of both CCW ((r)=0.6982 and CW ((r))=0.6732) commands (Fig. 3F).

However, activities of N1 and N2 during resting period, when the rat sat in the chamber for 6 min without seeing the water dish (without the visual cue) and thereby with no intention to control the BCI system, were significantly lower to those during T-WD trial period (Fig. 4D, E) (Fuster and Jervey, 1982). Many previous results presented that neuron units showed accelerating discharge during the delay while animals prepares for movement in a variety of tasks, including delay tasks (Kubota et al., 1974; Sakai, 1974; Niki and Watanabe, 1976; Fuster et al., 1982; Boch and Goldberg, 1989). Neural activities during T-NWD were intermediate between T-WD and resting periods. Thus, both neurons' activities were highly elevated during intentional control of the water dish, but they were suppressed during resting period. Therefore, stop commands observed during T-WD period and during resting period were generated by distinctively different levels of similar threshold values dictated by two neurons. Intermediate activity levels during T-NWD period may be considered as a very short resting period with anticipation for WD or it may be considered as an intermittent volitional wheel rotation periods to retrieve the water-containing region. Neural activities during FF, FW, FAR periods were quite variable among 16 rats and they were not significantly different among each other (Fig. 3D), suggesting that tight modulation of neural activities was not necessary in comparison to those during WD trial periods (Fig. 4E).

ACKNOWLEDGMENTS

This study was supported by grants to HCSHIN (Hallym Univ.-2008, MEST-Frontier research-2009-K001280, MKE-Industrial Source Technology Development Program-10033634-2009-11 & MEST-NRF-Priority Research Centers Program-2009-0094073).

REFERENCES

Boch RA and Goldberg ME (1989) Participation of prefrontal neurons in the preparation of visually guided eye movements in the rhesus monkey. *J Neurophysiol* 61:1064-1084.

Carmena JM, Lebedev MA and Crist RE (2003) Learning to control a brain-machine interface for reaching and grasping by primates. *PLoS Biol* 1:e2.

Chapin JK, Moxon KA, Markowitz RS and Nicolelis MAL (1999) Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. *Nature Neurosci* 2:664-670.

Fuster JM (1989) The prefrontal cortex: anatomy, physiology, and neuropsychology of the frontal lobe. 2nd ed. Raven Press, New York.

Fuster JM (1973) Unit activity in prefrontal cortex during delayed-response performance: Neuronal correlates of transient memory. *J Neurophysiol* 36:61-78.

Fuster JM and Jervey JP (1982) Neuronal firing in the inferotemporal cortex of the monkey in a visual memory task. *J Neurosci* 2:361-375.

Fuster JM, Bauer RH and Jervey JP (1982) Cellular discharge in the dorsolateral prefrontal cortex of the monkey in cognitive tasks. *Exp Neurol* 77:679-694.

Johan W, Christopher RS, Jerald DK, Pamela DB, Mark L, John KC, Jung K, S. James B, Mandayam AS and Miguel ALN (2000) Real-time prediction of hand trajectory by ensembles of cortical neurons in primates. *Nature* 408:361-365.

Jose MC, Mikhail AL, Roy EC, Joseph EO, David MS, Dragan FD, Parag GP, Craig S H and Miguel AL Nicolelis (2003) Learning to Control a Brain-Machine Interface for Reaching and Grasping by Primates. *PLOS Biology* 1:193-208.

Kennedy PR, Andreasen D, Ehirim P, King B, Kirby T, Mao and Moore M (2004) Using human extra-cortical local field potentials to control a switch. *J Neural Eng* 1:72-79.

Kubota K, Iwamoto T, and Suzuki H (1974) Visuokinetic activities of primate prefrontal neurons during delayed-

response performance. *J Neurophysiol* 37:1197-1212.

Lee U, Lee HJ and Shin HC (2007) Development of neuronal signal mapping method for 2D encoding-based brain-computer interface system. *Electronics Letters* 43:1408-1410.

Levine SP, Huggins JE, BeMent SL, Kushwaha RK, Schuh LA, Rohde MM, Passaro EA, Ross DA, Elisevich KV and Smith BJ (2000) A direct brain interface based on event-related potentials. *IEEE Trans Rehabil Eng* 8:180-185.

Lucas JW, Schiller JS and Benson V (2004) 2004 Summary health statistics for U.S. adults: national health interview survey. *Vital Health Stat* 10:60-62.

Mijail DS, Nicholas GH, Liam P, Matthew RF and John PD (2002) Instant neural control of a movement signal. *Nature* 416:141-142.

Musallam S, Corneil BD, Greger B, Scherberger H and Andersen RA (2004) Cognitive control signals for neural prosthetics. *Science* 305:258-262.

Niki H and Watanabe M (1976) Prefrontal unit activity and delayed response: relation to cue location versus direction of response. *Brain Res* 105:79-88.

Olson BP, Si J, Hu J and He J (2005) Closed-loop cortical control of direction using support vector machines. *IEEE Trans Neural Syst Rehabil Eng* 13:72-80.

Paxinos G and Watson C (1999) The Rat Brain in Stereotaxic coordinates. 4th ed. San Diego: Academic Press. pp 96-101.

Sakai M (1974) Prefrontal unit activity during visually guided lever pressing reaction in the monkey. *Brain Res* 81:297-309.

Serruya MD, Hatsopoulos NG, Paninski L, Fellows MR and Donoghue JP (2002) Instant neural control of a movement signal. *Nature* 416:141-143.

Shenoy KV, Meeker D, Cao S, Kureshi SA, Pesaran B, Buneo CA, Batista AP, Mitra PP, Burdick JW and Andersen RA (2003) Neural prosthetic control signals from plan activity. *Neuroreport* 14:591-597.

Taylor DM, Helms TSI and Schwartz AB (2002) Direct cortical control of 3d neuroprosthetic devices. *Science* 296:1829-1832.

Teuber HL (1972) Unity and diversity of frontal lobe functions. *Acta Neurobiol Exp (Wars)* 32:615-656.

Yang CR, Seamans JK and Gorelova N (1996) Electrophysiological and morphological properties of layers V-VI principle pyramidal cells in rat prefrontal cortex in vitro. *J neurosci* 16:1904-1921.