Brain electrophysiological activity correlates with temporal processing in rats

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ABSTRACT

In this study, we present electroencephalographic (EEG) recording data obtained in correlation with timing behavior in rats trained in a 30-s peak interval (PI) procedure. The distribution of lever press responses was found to be Gaussian, peaking at approximately 30 s: lever pressing behavior increased for 30 s then decreased after the reinforcement time. We recorded EEG activity in the hippocampus (hippocampal theta wave) and striatum during the task, and evaluated whether the EEG power correlated with the behavior pattern. We found that the striatum EEG, but not the hippocampal theta wave, showed a good correlation with the response pattern in the 30-s PI. This result suggests that striatum neurons fired more synchronously at the time of reinforcement, thus supporting a critical role for synchronization of firing of striatal neurons in regulating timing mechanisms.

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1. Introduction

Timing is everything in living animals. It is very important to study timing in order to understand animal behavior. Interval timing is the ability of animals to perceive and process durations in the seconds-to-minutes range (Buhusi and Meck, 2005). How do animals estimate the passage of time? Numerous human and animal studies have demonstrated that the brain is equipped with multiple time-measurement systems associated with interval-timing behavior.

The role of the hippocampus in temporal discrimination learning was initially explored by Meck et al. (1984). More recently, the hippocampus has become a primary target region in the study of the cognitive functions associated with temporal discrimination learning (Howard and Eichenbaum, 2013; Sakata, 2006; Yin and Troger, 2011). For example, in studies using the peak-interval (PI) procedure, rats with hippocampal lesions show a small leftward shift in their temporal discrimination function (Meck et al., 1984). Moreover, a frequency analysis of the hippocampal theta wave revealed an increase in power during a duration discrimination task compared with that observed in a simple reaction-time task (Sakata and Onoda, 2003). The hippocampal EEG theta wave is a large amplitude sinusoidal wave reflecting brain oscillation activity in the hippocampus (see Fujisawa and Buzsaki, 2011;

Fig. 1 for typical raw wave data). These results suggest that the hippocampus contributes to timing and the perception of time.

A variety of neuropsychological, functional neuroimaging and pharmacological studies suggest that the striatum, the major input structure of the basal ganglia complex, is an essential component of the neural networks involved in time perception. Neurons in the striatum are able to read time codes emitted by oscillator cells in the cortex (Matell et al., 2003). Impaired time perception has been found in patients with Parkinson's disease (PD), attributed to the presence of a dysfunctional dopaminergic striatal pacemaker in such patients (Dusek et al., 2012; Malapani et al., 1998).

The hippocampal formation sends projections to several structures, including the prefrontal cortex and ventral striatum. Both structures exhibit coherent theta rhythmicity. Previous studies have demonstrated that coherence between the striatum and hippocampus at the theta frequency is strong in the ventral/medial striatum and weak in the dorsal/lateral striatum (Berke et al., 2004). Pathways interconnecting the hippocampus and striatum are thought to use theta rhythms to transfer and coordinate neural representations in hippocampus–striatum circuits in relation to procedural maze tasks (DeCoteau et al., 2007). Should the hippocampus and striatum contribute to time perception, then the theta power of the hippocampus and striatum should increase during interval timing tasks.

The purpose of this study was to examine the variation in the hippocampus and striatum theta power related to temporal discrimination tasks, in particular during the peak-interval (PI) procedure. Among temporal discrimination tasks, the PI procedure is a

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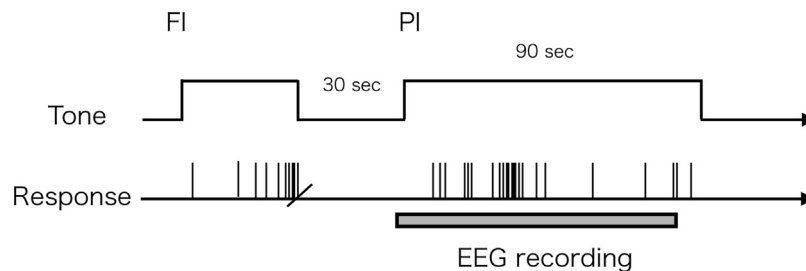
Fig. 1. The experimental procedures of the PI training and PI test sessions. The horizontal lines represent time. The square peaks on the "tone" axis indicate the presentation of pure tones. The vertical lines on the "response" axis indicate the lever response. The oblique lines indicate the supply of food pellets. The PI task consisted of two types of trials: FI trials (50%) and peak trials (50%). The rats were trained using a 30-s FI 30 schedule (FI), during which the first lever press that occurred 30 s after the beginning of the signal triggered the delivery of a food reward. PI sessions included probe trials in which the lever was extended for 90 s and the lever presses were not reinforced. In the test session, the EEG recording window started 4 s before stimulus onset and ended 80 s after stimulus onset in the peak trial.

powerful tool for studying timing in rats (Meck et al., 2008). In the PI procedure, the animals are trained to respond in the presence of a stimulus after a given amount of time has elapsed. Therefore, we hypothesized that the pattern of response in a peak procedure should correlate with theta waves in the hippocampus and striatum.

2. Methods

2.1. Animals

The subjects were six male Wistar rats (CLEA Japan, Inc., Osaka, Japan). Rats were 12 weeks old when the experiment began. Rats were housed in individual cages and kept on a 12:12-h light–dark cycle (lights on at 8:00 a.m.). Throughout the experiment, all rats were maintained at 85% of their *ad libitum* weight. Water was freely available throughout the experiment. The experiment was conducted in accordance with the guidelines published by the Japan Society for Animal Psychology and approved by the Committee of Experimental Animals at Hiroshima University (D08-6).

2.2. Apparatus

Behavioral training and EEG recording sessions took place in a standard operant chamber (ENV-007 CT; MED Associates, Inc., Georgia, VT, USA). According to the procedure, 45-mg food pellets (F0165; Bio Serv, Frenchtown, NJ, USA) were delivered in a cup was placed at the center of the front wall at the floor level. A food dispenser delivered the pellets to the cup with a click noise. A lever was positioned on the left side of the front aluminum wall. The chambers were equipped with a house light and a back wall-mounted speaker to provide tone stimuli. The experimental control and lever pressing data recording was performed using a PC running an in-house software program developed in Delphi (Borland Software Corporation). The chambers were housed in a soundproof, ventilated and electrically shielded room and monitored with a video camera (Mother tool CO. Model MTB-2081P).

2.3. Procedures

2.3.1. Initial training
In the initial preparation training session, the rats received 3 days of training for lever pressing for up to 60 pellets or 30 min. Next, the rats progressed through the following training sequence: (a) a continuous reinforcement (CRF) schedule for 60 pellets; (b) a fixed ratio 3 (FR3) reinforcement schedule; (c) an FR6 reinforcement schedule; (d) an FR10 reinforcement schedule for 60 trials per session. One training and testing session were conducted per day. Afterwards, rats were trained in the timing procedures described below.

2.3.2. Fixed-interval (FI) training
All rats received 15 sessions of a 30-s FI schedule in which a tone signal (2000 Hz, 80 dB) signaled the to-be-timed fixed interval. The sessions consisted of 60 trials, during which the first lever press that occurred 30-s after the beginning of the signal triggered the delivery of a food reward and terminated the tone signal for the duration of the variable intertrial interval (ITI) (average ITI 30 s, range 20–40 s).

2.3.3. Peak-interval (PI) task training
After the FI training, the rats received 50 sessions of PI task training (Fig. 1). The PI session consisted of 60 trials, of which 50% were FI trials and 50% were PI trials. During the PI trials, the lever was extended for 90 s and the lever presses were not reinforced. The peak trials and FI trials were randomly intermixed with the restriction that no more than four peak trials occurred consecutively. As in the FI training, the trials were separated by variable ITIs (average 30-s, range 20–40 s). Afterwards, the rats underwent surgery for electrode implantation.

2.4. Surgery

The electrode implantation procedure was similar to that used in a previous study (Sakimoto et al., 2013a, 2013b). The electrodes were implanted stereotaxically into the hippocampal region (3.5 mm posterior from bregma, 1.5 mm lateral from the midline and 2.5 mm beneath the skull surface) and the striatum region (0.7 mm anterior from bregma, 2.8 mm lateral from the midline and 4.6 mm beneath the skull surface). Other electrodes were implanted into the frontal cortex, parietal cortex, primary auditory cortex and cerebellar cortex. The EEG data for the frontal cortex, parietal cortex and primary auditory and cerebellar cortex are not shown due to the presence of artifacts. The data obtained from the hemisphere with fewer artifacts and less noise in each rat were used for data analysis. The electrodes were connected to a nine-pin connector (Amphenol, Wallingford, CT, USA) and fixed with dental cement to the screws and skull. After surgery, each rat was given a recovery period of at least 1 week. After the recovery period, the rats were re-trained in the PI task. The rats' EEG data were recorded throughout the PI task following the extinction schedule.

2.5. Test session and extinction session

After the recovery period, the rats were re-trained in the PI task. The EEG data were then recorded during the peak trials for three PI test sessions. Finally, rats were also tested in three extinction sessions similar to the 30-s PI sessions, except no reinforcement was delivered. All parameters were the same as those used during training.