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Activity of Striatum and Globus Pallidus of Rats during Elevated Body Swing Test

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ABSTRACT

Chronic multi-channel single unit recordings were done simultaneously in both striatum (STR) and globus pallidus (GP) of rats while rats were repeatedly performing elevated body swing test (EBST), which was consisted of 2 major parts: moving above ground (F) and moving on the ground (G). F had 2 parts: flying with movement (FM), flying without movement (FS). G had 4 behavioral segments: safety seeking (GS), resting (GR), moving head (GH) and exploring movement (GM). Frequency, duration and direction of head movement were also determined during the EBST. The results showed that the GS was the shortest and the GR was the longest. The averaged percentage distributions of sub-periods of the EBST were very stable for 2 weeks. Frequency and duration of leftward head shift (HS) were similar to those of rightward HS. Neural activities of both FM and GR sub-periods were much stronger than those of other sub-periods. During FM, GR and GM sub-periods GP neurons showed significantly stronger activities than STR neurons did. These neuronal firings were stable for 2 weeks. Neural activities of each nucleus during HS were much stronger in FM sub- period than in ground sub-periods. GP neuron's firing rates were also much stronger than STR neuron's activities during HS. Activities during rightward HS were not significantly different from those during leftward HS. The stability of the sub-behavioral periods of the EBST and the neural activities of STR and GP during each sub- behavioral period further suggests that the EBST may be an excellent behavioral tool for the study of various abnormalities involving basal ganglia.

Key words: Basal ganglia, striatum, globus pallidus, elevated body swing test

INTRODUCTION

The basal ganglia is a group of subcortical nuclei involved in motor control, cognition, and emotion. Basal ganglia disorders are manifested by abnormal movement and a number of neuropsychiatric disorders. Basal ganglia nuclei are organized into sensorimotor, associative, and limbic territories based on their connectivity and function. The caudate nucleus, putamen, and subthalamic nucleus comprise the input nuclei of the basal ganglia. The internal segment of globus pallidus (GP) and substantia nigra reticu-

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lata are the output nuclei. The input and output nuclei are interconnected by direct and indirect pathways. The cerebral cortex, basal ganglia, and thalamus communicate with each other via closed (segregated) parallel as well as open (split) loops.

Dysfunctions of the basal ganglia-thalamocortical circuits are associated with a variety of movement abnormalities, e.g., akinesia (poverty of movement), muscular rigidity and tremor in Parkinson's disease, and involuntary choreatic movements in hemiballismus and Huntington's chorea. Parkinson's disease and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinsonism result from a loss of doamine within the striatum (STR) (Burns et al., 1983; Langston et al., 1984)) leading to dramatic changes in the firing rate and patterns of single neurons in the basal ganglia (Bergman et al., 1994; Filion and Tremblay 1991; Miller and DeLong 1987; Rothblat and Schneider 1993) and to reduced specificity of pallidal neurons (Filion et al., 1988; Miller and DeLong 1988; Tremblay et al., 1989).

Elevated body swing test (EBST) has been used as a reliable behavioral test in 6-OHDA-induced hemiparkinsonian rats (Baluchnejadmojarad and Roghani, 2004; Borlongan and Sanberg, 1995; Borlongan et al., 1995; 1996; Roghani et al., 2002). This EBST is a pure behavioral test that can evaluate unilaterally lesioned animals in a drug-free state, reflecting a more natural response of the animals to neurotoxin lesion. However, there has been no attempt to monitor neural activities in the basal ganglia while animals performing the EBST.

In this study, to determine the behavioral correlations of neural activities during the EBST we carried out simultaneous many single unit recordings from rats implanted with microwire electrodes in STR and GP. The EBST behavior was analyzed and six behavioral sub-segments were identified. Frequency, duration and direction of head movement were also determined.

MATERIALS AND METHODS

All experiments were approved by the Hallym University Animal Care and Use Committee (HUACUC). Rats (Sprague Dawley, body weight= 250～280 g) were succeeded in HUACUC. The environment of breeding room was maintained at stable condition (23±2^{o}C and relative humidity was 55±10%). Artificial lighting was automatically maintained 12 h per d. Animals were housed 5 per cage with food and water was available ad libitum. They were transferred to laboratory just before electrode implantation surgery.

Rats (n=10) were anesthetized (Ketamine, Yuhan, Korea, 100 mg/kg and rompun (Bayer Korea, Korea, 5 mg/kg, i.m.)) for implantation of microwire electrodes. During the surgery, supplemental dosage of ketamine was injected as required to maintain an appropriate depth of anesthesia. They were transferred to a stereotaxic apparatus and fixed as prone position. Subcutaneous lidocane injection was given on the scalp before midline incision. After removal of the skin and soft tissue, a relatively large craniotomy (2～3 mm diameter) was made bilaterally over the basal ganglia. As a preparation, 6 jeweler's screw holes were drilled and 6 screws were turned in as an anchor to the skull. Care was taken not to depress the dura and surface of the brain. And then, dura mater was removed. The left and right basal ganglia were identified according to rat brain atlas (Paxinos & Watson, 1998). For each hemisphere, two bundles of multi-wire recording electrodes (4 electrodes for each bundle, tungsten microwire, A-M systems, USA, 75μm diameter, teflon-coated, 2.2 mm interval between bundles) were positioned perpendicular to the cortex. Then it was lowered targeting the STR (3.2 mm lateral from midline, 0.2 mm anterior from bregma, 4 to 6 mm from brain surface) and GP (3.2 mm lateral from midline, 1.3 mm posterior from bregma, 6 to 7 mm deep from brain surface) with hydraulic micro mover (Narishige, Japan). A head stage plug was used to connect the implanted electrodes to a preamplifier whose outputs were sent to a Multi- Neuronal Acquisition Processor (MNAP, Plexon Inc., Dallas, TX, USA) for online multi-channel spike sorting and data acquisition. A maximum of four extracellular single units per micro-wire and total maximum of up to 32 units per experiment could be discriminated in real time using time-voltage windows and a principal component-based spike sorting algorithm (Nicolelis et al., 1993). Autocorrelation histograms were also generated to verify the individuality of the single unit firing. Correct positioning of the electrode bundles was verified by histological examination under

light microscope after sacrificing the animal. Head stage holding the electrode bundles were cemented altogether with dental resin to the pre-screwed anchors. After surgery, each rat was transferred to a previously sterilized cage. At least two weeks of recovery period was given before experiment. During this period, water and food were supplied ad libitum and they were intensively cared in the HUACUC. Body weight was regularly checked to monitor the level of dehydration and health status. After recovery each rat was moved into experimental cage, and the electrode head stage was installed to check the existence of neuronal units, and templates of units were prepared and saved separately for each rat in the hard disk for later use in experiment.

Elevated body swing test (EBST) was done for measuring behavioral integrity while simultaneously recording extra-cellular single neuron activities from implanted micro-wire electrodes in the STR and GP. The EBST was conducted in a box (40×40×40 cm). Initially, the rat was habituated in the box for 5 min. The rat was elevated an inch above the ground by holding its tail for 20 sec and then it was grounded for 60 sec. This sequence of elevation and grounding was repeated for 20 min. All behaviors were taped with camera and later frame by frame analysis was done to correlate neural activities from both nuclei. This behavioral sequence was subdivided into 6 parts; FM (flying-movement), FS (flying stop), GS (ground, safety seeking), GR (ground rest), GH (ground head movement), GM (ground exploratory movement). The duration of each period was measured. The direction, frequency and duration of head rotation (more than 10^{o}C swing from midline) were also recorded. Neural activities of STR and GP during EBST were simultaneously recorded for 2 weeks (30 min, 3 h. 12 h, d 1, 2, 4, 8 and 2 weeks). All data were presented as mean ±SEM.

RESULTS

When rats were at ground during the EBST, the GS was the shortest (4.86±1.2% of whole period) and the GR was the longest (32.75±6.4%, Table 1). The averaged percentage distributions of sub-periods of the EBST were very stable for 2 weeks. Frequency of head rotation to left was also similar to that of right (during FM period: left: 4.37±1.0, right: 3.78±0.7; during GH and GM sub-periods: left: 11.36±3.2, right: 9.01±3.0). Duration of head rotation to left was similar to that to right (during FM period, left: 2.50±0.5%, right: 2.87±0.5%; during GH and GM sub-periods: left: 3.77±0.5%, right: 3.29±0.5%).

In the right hemisphere, neural activities of both FM (STR: 64.47±8.0 Hz, n=92; GP: 154.46±13.2 Hz, n=103, with 10 rats, Fig. 1) and GR (STR: 57.39±5.4 Hz, GP: 92.35±9.3 Hz) sub-periods were much stronger than those of other sub-periods (STR: 18～33 Hz, GP: 23～44 Hz). During FM, GR and GM sub-periods GP neurons showed significantly (*$p<0.01$) stronger activities than STR neurons did. Although neural activity of each nucleus during GM period was significantly (*$p<0.01$) weaker than that during either FM or GR periods, it was significantly ($p<0.05$) stronger than activities during other sub-periods (FS, GS and GH). Activities recorded during FS, GS and GH sub-periods were not significantly different among them in each nucleus. These neuronal firings were not altered till 2 weeks after saline injection to the STR of right hemisphere (Fig. 2). Neural activities of each

Table 1. Percentage distribution of EBST sub-periods for 2 weeks in normal rats

	FM	FS	GS	GR	GH	GM
Control	16.34±2.5	8.79±2.2	4.86±1.2	32.75±6.4	16.77±2.8	22.89±3.6
1 d	18.39±4.0	6.37±1.6	4.98±1.5	31.59±4.2	16.98±3.9	21.71±4.0
2 d	17.49±3.2	7.94±2.0	4.99±1.3	30.78±4.9	18.20±4.2	20.60±4.4
4 d	19.02±4.3	5.60±1.4	4.66±1.0	31.70±5.0	17.60±3.6	21.49±4.9
8 d	18.38±4.0	6.57±2.1	5.02±1.3	32.59±6.4	16.49±4.4	22.59±5.0
2 week	17.60±3.7	7.70±2.2	4.17±1.2	32.49±6.3	15.70±3.2	23.56±4.9

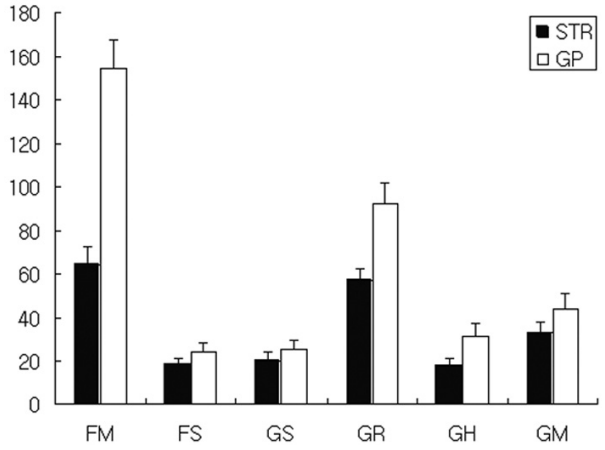

Fig. 1. Firing rates of STR and GP neurons during 6 sub-periods of EBST. Y axis: spikes/sec. STR: striatum, GP: globus pallidus. FM: flying-movement, FS: flying stop, GS: safety seeking at ground, GR: rest at ground, GH: head movement at ground, GM: exploratory body movement at ground.

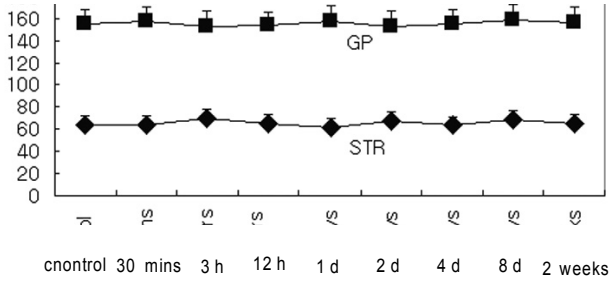

Fig. 2. Stability of activities of STR and GP neurons during FM sub-period for 2 weeks. Y axis: spikes/sec. STR: striatum, GP: globus pallidus.

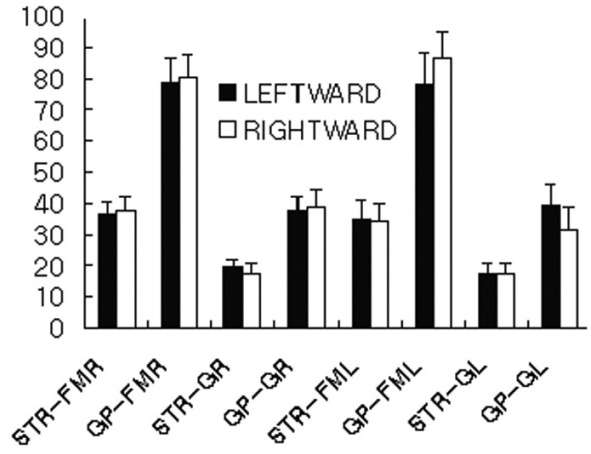

Fig. 3. Firing rates of STR and GP neurons in both hemispheres during head rotation movement to either right or left directions in FM sub-period and in ground (G) sub-periods. R of the FMR stands for right hemisphere. L of the FML stands for left hemisphere. R of the GR represents right hemisphere. L of the GL represents left hemisphere. Y axis: spikes/sec. STR: striatum, GP: globus pallidus.

nucleus during head rotations were much stronger (*$p < 0.01$) in FM sub-period than in ground sub-periods (Fig. 3). GP neuron's firing rates were also much stronger (*$p < 0.01$) than STR neuron's activities during head rotations. Activities during rightward rotations were not significantly different from those during leftward rotations.

DISCUSSION

In the current study our EBST was distinctively different from other studies employing EBST (Baluchnejadmojarad and Roghani, 2004; Borlongan and Sanberg, 1995; Borlongan et al., 1995; 1996; Roghani et al., 2002). Previously EBST simply involved elevating the animal by handling its tail and recording the frequency and direction of the swing behavior. In the current study the EBST had additional ground behavior. The 30 sec elevated period was subdivided into two, FM and FS. The 60 sec ground behavior was further sequentially classified as GS, GR, GH and GM sub-periods. This was possible by frame-by-frame analysis of recorded videos. Frequency, duration and direction of head movement were analyzed both elevated and ground periods. The percentage distribution of each sub-periods of the EBST as well as duration and frequency of head rotations were extremely stable for several weeks. This behavioral stability was also paralleled with stable firings of both STR and GP neurons in control rats during different sub-periods of the EBST.

None of previous studies employed EBST had recorded neural activities from either STR or GP of awake, behaving rats. Furthermore, we have also simultaneously recorded neural activities from both nuclei during EBST and subsequent ground behavioral period. Striatal neuron's averaged firing rates were much higher than those observed in previous chronic single unit recordings in awake, conscious rats. Kish et al., (1999) reported the mean rates of 1.1 Hz and 4.3 Hz for striatal neurons recorded in similar dorsal striatum as in our study during quiet rest. Chen et al., (2001) also reported fairly low firing rate (0.76±0.16 Hz) in awake, rat. In the

current study spontaneous firing rate of striatal neuron was about 60 Hz during either GR or FM sub-periods. Activity rate for other sub-periods were around 20 Hz. Wang and Rebec (1993) have reported mean firing rates during quiet rest in freely moving rats ranging from 2.5 Hz for motor-related neurons to 18.5 Hz for non-motor-related neurons. Firing rate values obtained by Wilson and Groves (1981) for 21 medium spiny neurons in urethane anesthetized rats ranged from 0.24 to 32.7 Hz with a mean of 5.54 Hz and a standard deviation of 7.38. Historically, firing rates of striatal neurons in anesthetized animals have been reported as ranging between 1 and 2 Hz. The higher firing rates observed in this study may be caused by the repetition of EBST, where the rat had performed different sub-behavioral segments from FM to GM for 20 min.

Sardo et al., (2002) reported that GP neurons had sustained discharge, with a mean firing rate of 25.01±9.21 Hz. Spontaneous rates of discharge of pallidal neurons varied among different studies (19 ±1 Hz, Querejeta et al., 2001; 22.14±1.42 Hz, Ni et al., 2000) in anesthetized rats. Thus, GP activity is generally higher than STR activity in anesthetized rats. In our study with behaving conscious rats, this trend is also quite evident during FM and GR sub-periods. The strongest firing was observed during FM sub-period (154 Hz) and less strong response during GR sub-period (92 Hz). However, during other sub-periods, spontaneous responses ranged from 23 to 44 Hz. Thus, the firing rate of GP neurons appears in the right range if we consider the tendency of increased activity in the absence of anesthesia. Since GP and STR activities were recorded simultaneously in this study, we believe that the strong STR activity compared to previous results was occurring also in physiological ranges during the repetition of EBST and subsequent ground period.

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