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Research article

Modulatory effect of subthalamic nucleus on the development of fatigue during exhausting exercise: An in vivo electrophysiological and microdialysis study in rats

Dalei Wang, Xiaoli Liu and Decai Qiao ✉
College of Physical Education and Sports, Beijing Normal University, Beijing, 100875, China

Abstract
The purpose of the study was to investigate the modulatory effect of changes of subthalamic nucleus (STN) activity on the development of central fatigue during exhausting exercise, and reveal the possible mechanism that might affect STN activity from the perspective of neurotransmitters. Rats were randomly divided into electrophysiology and microdialysis study groups. For electrophysiological study, electrical activity in sensorimotor cortex and STN were simultaneously recorded before, during and 90min after the exhausting exercise. For microdialysis study, extracellular fluid of STN was continuously collected with a microdialysis probe and glutamate (Glu), gamma-aminobutyric acid (GABA) levels were subsequently detected with high performance liquid chromatography (HPLC). The behavioral studies showed that rats ran well initiatively with the treadmill exercise in the beginning, 45±11.5min later, movement capacity reduced obviously (which was termed as 'early fatigue'). Correspondingly, STN activity increased significantly compared with rest condition ($p < 0.05$), while, cortex activity decreased significantly ($p < 0.05$). Subsequently, rats continued their exercise with minor external stimulation till exhaustion. Cortex activity reached the minimum value under exhaustion condition, while STN activity changed insignificantly ($p > 0.05$). For microdialysis study, the dynamic change of Glu/GABA ratio was consistent with the change of STN activity during the development of 'early fatigue' rather than the development of exhaustion. In conclusion, the present study shows that, the development of the cortex fatigue during exhausting exercise consists of two phases, 'early fatigue' and exhaustion. Our results suggest that, dynamic changes of STN activity are closely relevant to the development of 'early fatigue' rather than exhaustion, and the changes of STN activity during the development of 'early fatigue' might be partly related to the variance of Glu and GABA levels in STN extracellular fluid.

Key words: Subthalamic nucleus, central fatigue, exhausting exercise, electrophysiology, microdialysis.

Introduction

Exercise-induced fatigue is a complicated phenomenon. Generally, it can be divided into two types: peripheral fatigue which induced mainly by the peripheral factors, for example, the depletion of energy in muscle, and central fatigue which induced mainly by the cerebral perturbations during exercise (Chaudhuri and Behan, 2000). Fatigue should be acknowledged as a complex phenomenon influenced by both peripheral and central factors. However, for the complexity of fatigue as well as the complexity of the brain, physiological investigations have focused mainly on the relationship between fatigue and circulatory, metabolic, muscular, and nutritional factors (Nybo and Secher, 2004). The relationship between fatigue and neurophysiological changes, has received little scientific attention. Recently, lots of experiments have verified the effect of central nervous system (CNS) on fatigue, cerebral metabolic and neurohumoral alterations during strenuous exercise may result in the impairment of cortex activity, and this may lead to the failure of CNS sustaining adequate central drive to spinal motoneurons (Davis and Bailey, 1997). Under some circumstances, failure to drive the motorneurons adequately as a consequence of neurophysiological alterations seems to play a dominant role in modulating the development of fatigue (Gandevia, 2001).

However, the exact mechanism for CNS modulating fatigue during strenuous exercise still remains to be fully understood. Motor cortex is one of the most important structures for movement control, and the impairment of motor cortex activity may directly affect the exercise performance. The activity of motor cortex is affected by complicated factors. The basal ganglia (BG) consists of six extensively interconnected nuclei, the caudate nucleus, putamen, globus pallidus, substantia nigra, amygdala (archistriatum) and subthalamic nucleus (STN). STN is one of the most important nuclei in BG, changes of STN activity play an important role in modulating excitability of cortex, as well as the initiation and termination of voluntary movement (Nambu et al., 2002). Aron et al. (2007) reported that STN was significantly activated when motor plan was canceled. Patients with Parkinson disease (PD) is characterized with abnormal burst firing pattern in STN, combined with some cardinal motor signs such as bradykinesia, rigidity, and postural instability, thus, STN has been regarded as one of the most important nuclei of 'brake' system (Jahanshahi et al., 2000). Due to the critical modulating effect of STN on motor cortex activity, we would predict that, STN might take part in modulating the exercise performance and fatigue development by exerting inhibitive effect on cortex activity during strenuous exercise. In the present study, we simultaneously monitored the variance of neuron activity in STN and motor cortex during exhausting exercise. On the other hand, since glutamate (Glu) and γ-aminobutyric acid (GABA) play the dominant regulating effect on STN activity, changes of Glu and GABA levels in STN extracellular fluid were observed by a microdialysis study as well. The purpose of the study is to 1) investigate the modulatory effect of changes of STN activity on the development of central fatigue during exhausting exercise; 2) reveal the

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possible mechanism that might affect STN activity from the perspective of neurotransmitters.

Methods

Experimental animals

Male Wistar rats (Beijing Vital River Laboratory Animal Technological Company, Beijing, China), weighing 290±20g, were used for all experiments. All the rats were randomly divided into two groups: electrophysiology study group and microdialysis study group. Animals were maintained on a 12h light/dark cycle and given food and water *ad libitum*. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Surgery

For electrophysiological recording, the rats were anesthetized with pentobarbital sodium (50mg/kg, Sigma) and placed on a stereotaxic frame. After the hair above the skull had been removed, an incision was made along the midline and a burr hole was drilled to implant a concentric bipolar electrode in the left STN, coordinates relative to bregma were AP-3.6mm, L-2.5mm, and D-7.6mm (Paxinos and Watson, 1997). 1 stainless steel screw (1mm diameter) was positioned above sensorimotor area (AP-1.8mm, L-2mm, D-0.5mm) for electrocorticogram (ECoG) recording. Another stainless steel screw was fixed to the skull above the cerebellum (AP-10.0 mm, L-0 mm, D-0.5 mm) as the reference. For microdialysis study, a cannula (4.15.IC, Microbiotech/se AB, Sweden) with replaceable inner guide was positioned 1 mm above the left STN. The gaps surrounding the electrode/cannula were filled with softened paraffin. 3 holes were drilled surround the coordinates designed for the electrode/cannula, screws were secured in each of these holes and fixed with dental cement. Animals were allowed at least 5 days to recover from the surgery. 5 days later, the rats were subjected to adaptive exercise with progressive loading.

Exhausting exercise protocol

7 days after the surgery, rats that were able to sustain 30min of exercise (20m/min) continuously were selected for the next experiments. The electrophysiology/ microdialysis study was carried out the next day. Progressive loading treadmill exercise protocal was modified from the Bedford treadmill exercise program, and the loading was classified into 3 stages: stage I: 10m/min, 15min; stage II: 15m/min, 15min; stage III: 20m/min, till exhaustion (Figure 1-A) (Yang et al., 2009).

Simultaneous recording of ECoG in sensorimotor cortex and LFPs in STN

ECoG in sensorimotor cortex was recorded from the screw above the sensorimotor cortex. Local field potentials (LFPs) in STN were recorded from the concentric bipolar microelectrode in bipolar modes. Screw above the cerebellum was used as the reference. Before exercise, all the electrodes were connected to a miniature source following multi-channel JFET headstage which was mounted on the animal's head so as to reduce the movement artifacts. The signals were then inputted to the main amplifier (EX4-400, Dagan Corporation, USA) through a soft cable. In order to eliminate the electromagnetic interference generated from the electric motor of the working treadmill (HangZhou DuanShi, DSPT-208, China) during the exercise, the electric motor was wrapped with the copper mesh, and then the copper mesh was connected to the ground interface of the main amplifier. Raw signals were amplified (×200), band-pass filtered (0.1-100Hz) by the main amplifier and sampled on-line at 512Hz (PowerLab 8/30, AD Instrument, Australia). Dynamic changes of ECoG and LFPs were continuously recorded under rest, exercise, exhaustion, and recovery conditions, data were stored on a PC for offline analysis (Figure 1-B).

Microdialysis experiments

The microdialysis probe (MAB4.15.1, Microbiotech/se AB, Sweden) was implanted through the guide cannula and was perfused with artificial cerebrospinal fluid (aCSF) (126 mM NaCl, 2.4 mM KCl, 1.1 mM $CaCl_2$, 0.85 mM $MgCl_2$, 27.5 mM $NaHCO_3$, 0.5 mM Na_2SO_4, 0.5 mM KH_2PO_4, pH 7.0) at a flow rate of 2μL/min driven by a microinjection pump (MD-0250, Bioanalytical Systems, USA). To limit tissue damage and keeping in mind the small dimensions of the STN, we used a very small microdialysis probe (diameter of 0.2 mm and a membrane length of 1 mm) (Ampe et al., 2007). After equilibrating for 90 min, the brain microdialysate was collected every 15min by a refrigerated fraction collector (MAB 85,

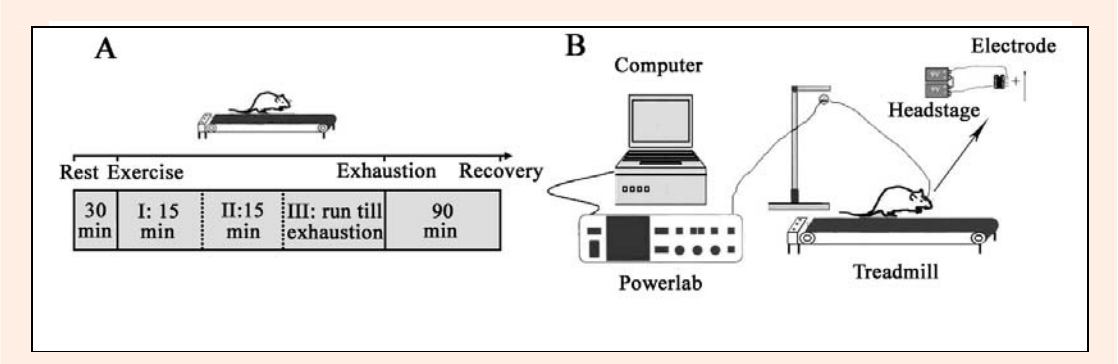

Figure 1. Schematic illustration of exhausting exercise protocol and ECoG/LFPs recording. A, exhausting exercise protocol. B, ECoG/LFPs recording during exhausting exercise.

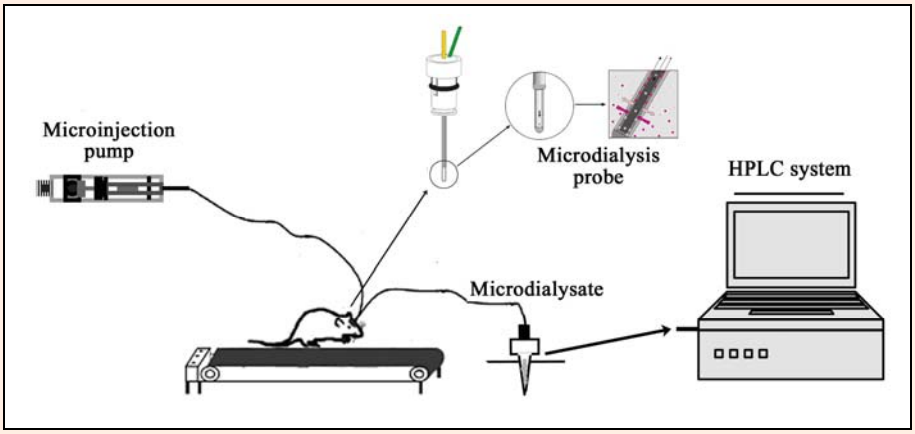

Figure 2. Schematic illustration of continuous collection of microdialysate during exercise and neurochemical detecting system

Microbiotech/se AB, Sweden) before, during and after exhausting exercise (Figure 2).

After electrophysiology/microdialysis experiments, Nissl staining was processed, all recording sites in the brain were verified by light microscopy (Figure 3), and data from the wrong located rats were not used.

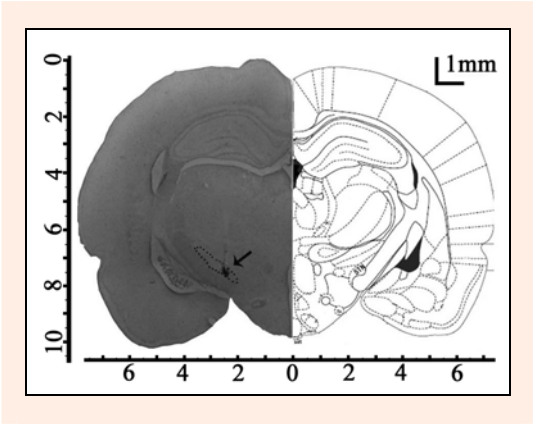

Figure 3. Histological confirmation of electrode/microdialysis probe placement.The electrode/ microdialysis probe was planted in the left STN (arrow) under stereotaxic conditions (AP-3.6mm; L-2.5mm; and D-7.6mm. Scale bar=1mm.).

Detection of Glu and GABA in microdialysate

Concentrations of Glu and GABA in the microdialysate samples were detected by high performance liquid chromatography (HPLC) using a fluorescence detector (LC-10AT vp plus, Shimadzu, Japan) after precolumn derivatization with OPA-β-mercaptoethanol. The mobile phases were (A) 0.1 mol/L KH_2PO_4 buffer (pH 6.6): methanol=65:35 (v/v) and (B) 0.1 mol/L KH_2PO_4 buffer (pH 6.6): methanol=10:90 (v/v). All HPLC chemicals were obtained from Sigma. Separation was achieved on a C18 column (5 μm, 4.6×250 mm, Shimadzu, Japan). 30s after mixing the microdialytic samples (30μL) and derivatization reagent (15μL), 20 μL of the reaction mixture was injected into the column and separated with a gradient (EX: 357 nm; EM: 455 nm).

Data presentation and statistical analyses

According to the results of behavioral studies, all the exercise procedure was divided into several stages. After experiment, electrophysiological data were analyzed offline with Chart5 (AD Instrument, Australia) and NeuroExplorer4 (Plexon, American) software. Epochs of 30s duration ECoG/LFPs raw data of each stage were selected to analyze the power spectrum density and power of different frequency bands by applying Fast Fourier Transform (FFT; Welch's method; FFT size: 512). Otherwise, 30s epoch of ECoG and LFPs signals was extracted every 15min from all the procedure to calculate the gravity frequency (f_g) by the following formula:

$$f_g = \frac{\sum_{f=f_1}^{f_2} (P(f) \times f)}{\sum_{f=f_1}^{f_2} P(f)}$$

Within which, f_1 to f_2 is the frequency range; $P(f)$ demonstrates power value that the corresponding frequency (f) contains. Neurotransmitter concentrations were calculated by comparing peak areas with standard solutions. Values were expressed as percentages of baseline levels to minimize the difference between subject variations. All the data were presented as Mean ± SD ($\overline{x}$ ±s). A one-way ANOVA with repeated measures was performed for the analysis of the effects of exhausting exercise on f_g and Glu, GABA concentrations. For statistical analysis of frequency bands power of different stages, one-way analysis of variance (ANOVA) was used and $p < 0.05$ were considered to be statistically significant.

Results

Behavioral studies

Behavioral study showed that, rats adjusted themselves well to the treadmill exercise in the beginning. 45±11.5min later, the exercise capacity of the rats decreased and showed difficulty in maintaining the preconditioned speed, we term this period as 'early fatigue'; If they were given slight sound, light or direct current stimulation when 'early fatigue' occurred, the rats might catch up with the preconditioned speed again till

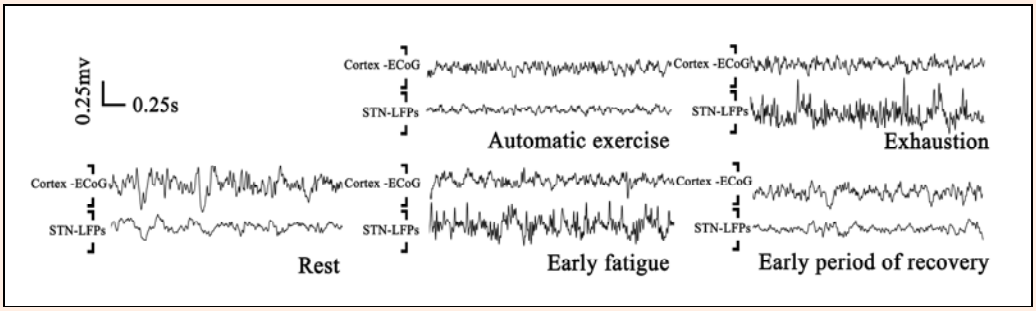

Figure 4. Raw data trace of ECoG and LFPs under different conditions.

exhaustion. Criteria of exhaustion were: the running posture changed into prostrate-style from stomp-style; stay in the rear part of the treadmill stationaryly and the light/sound/direct current stimulation could not keep it running again. According to behavioral study, the whole procedure of electrophysiologic/microdialysis experiment was divided into 5 conditions: rest condition; automatic exercise condition (15min after the start of exercise); 'early fatigue' condition; exhaustion condition and early period of recovery condition (30min after exhaustion), raw trace of electrophysiological signals under different conditions are as shown in Figure 4.

Dynamic changes of sensorimotor ECoG and STN LFPs

We calculated the power spectrum density (PSD) (Figure 5-A, 6-A) of different conditions. ECoG and LFPs wave can be divided into 5 frequency bands: δ (0.8-3.9Hz), θ (4-7.9Hz), α (8-12.9Hz), β (13-30Hz) and γ band (>30Hz). δ and θ band are slow waves, β and γ band are fast waves. The power of different frequency bands under different conditions were also calculated (Figure 5-B, 6-B), power of rest condition was regarded as baseline, and the power of other conditions were normalized versus the rest condition so as to eliminate the individual differences.

As shown in Figure 5-A and 6-A, two kinds of oscillations activity in sensorimotor ECoG and STN LFPs were significant: 1-2 Hz low frequency oscillation activity and 6-8Hz oscillation activity. For sensorimotor ECoG, compared with rest condition (Figure 5-B), power of θ band under automatic exercise condition decreased significantly ($p < 0.01$), however power of α band increased ($p < 0.05$); under 'early fatigue' condition, there was an increase in the power of δ band ($p < 0.05$); under exhaustion condition, a significant ($p < 0.05$) increase of δ band power was seen, whereas no significant changes were seen under early period of recovery condition ($p > 0.05$). In STN LFPs, compared with rest condition, there were significant increases in power of δ, θ, α and β bands under automatic exercise condition, especially in power of slow δ band ($p < 0.01$). Under 'early fatigue' condition, power of δ, θ bands ($p < 0.05$), α and β bands ($p < 0.01$) all increased significantly; under exhaustion condition, significant ($p < 0.05$) increases in power of α and β bands were seen; under early period of recovery condition there were no significant changes ($p > 0.05$).

Dynamic changes of gravity frequency in sensorimotor ECoG and subthalamic LFPs

f_g may reflect the activity of neuron assembles around the recording electrode (Klimesch, 1999). Transitioning to low frequency implies the decrease of neuron activity while transitioning to high frequency may reflect the increase of neuron activity. As shown in Figure 7, in the beginning, f_g of ECoG raised firstly and then declined, at 75min time point the f_g was significantly lower than that of the rest condition ($p < 0.05$), at the same time, rats showed decrease in exercise capacity. After stimulating with slight sound/light/electrical stimulation, the rats might catch up with the preconditioned speed again, and the f_g raised correspondingly. Then, f_g declined gradually and reached the minimum value when exhaustion occurred ($p < 0.05$), and gradually recovered again during

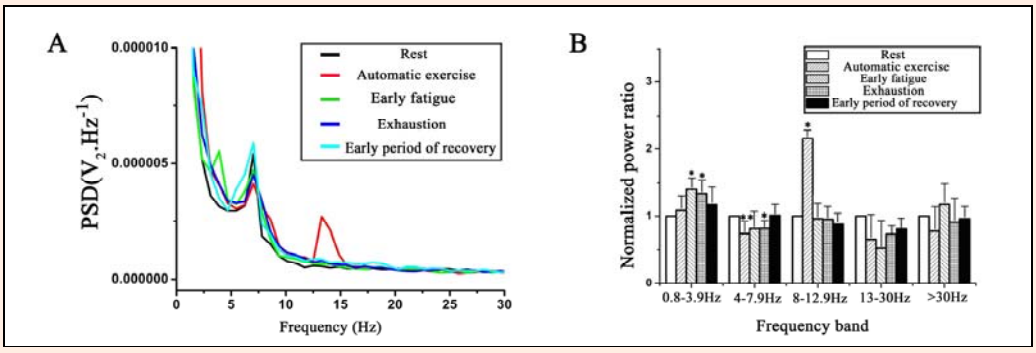

Figure 5. Changes of electrocorticogram in the sensorimotor cortex. A, Power spectral density of all conditions. B, Normalized spectral changes divided into frequency bands. *p < 0.05, **p < 0.01 *vs* rest condition.

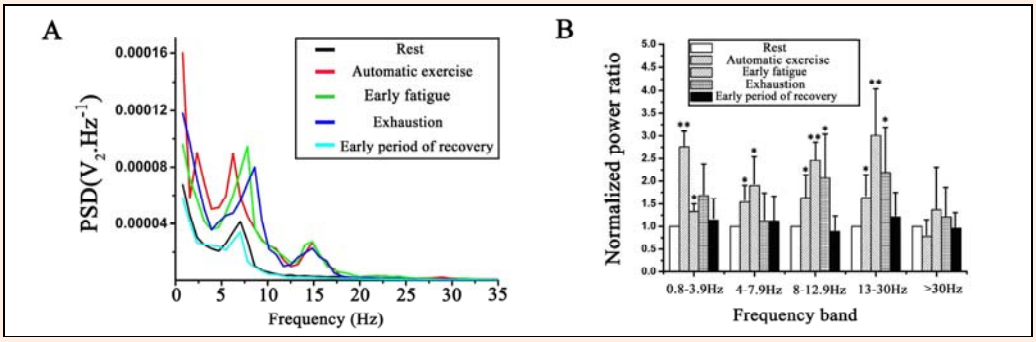

Figure 6. Dynamic changes of local field potentials in the STN. A, Power spectral density of all conditions. B, Normalized spectral changes divided into frequency bands.* p < 0.05, ** p < 0.01 *vs* rest condition.

recovery period. Generally, f_g of LFPs changed inversely, and reached the maximum value at 75min time-point ($p < 0.05$, compared with the rest condition), changes at other time points were not significant ($p > 0.05$).

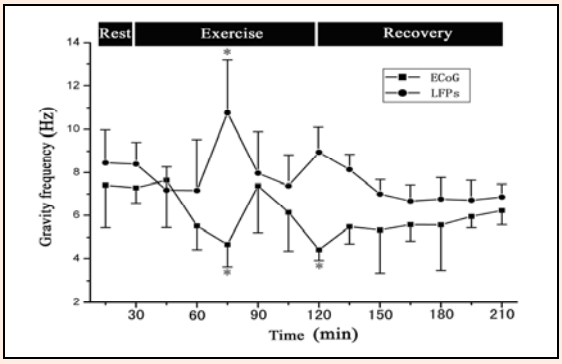

Figure 7. Dynamic changes of gravity frequency of electrocorticogram in the sensorimotor cortex and local field potentials in the STN * p < 0.05 *vs* rest condition.

Dynamic changes of Glu and GABA levels in STN extracellular fluid

Concentrations of Glu and GABA in STN extracellular fluid all decreased significantly with the progress of exhausting exercise (Figure 8). Glu/GABA ratio was always used as the parameter to reflect the activity of neurons (Xu et al., 2007). In our study, Glu/GABA decreased in the beginning, 45min later Glu/GABA increased significantly ($p < 0.05$) and gradually decreased again. When exhaustion occured, another peak of Glu/GABA was found, and 15 after exhaustion, Glu/GABA reached the minimum value and then increased again (Figure 9).

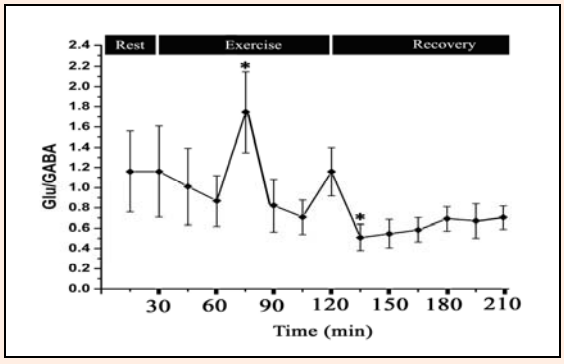

Figure 9. Ratio of glutamic acid/gamma-aminobutyric acid concentrations in STN extracellular fluid with the development of fatigue. * p < 0.05 *vs* rest condition.

Discussion

Effect of the subthalamic nucleus on the ECoG activity during exercise

BG consists of a group of nuclei and plays an important role in movement control. Previous concept considered that, the process of movement information in BG was accomplished by two main pathways: 'direct' pathway

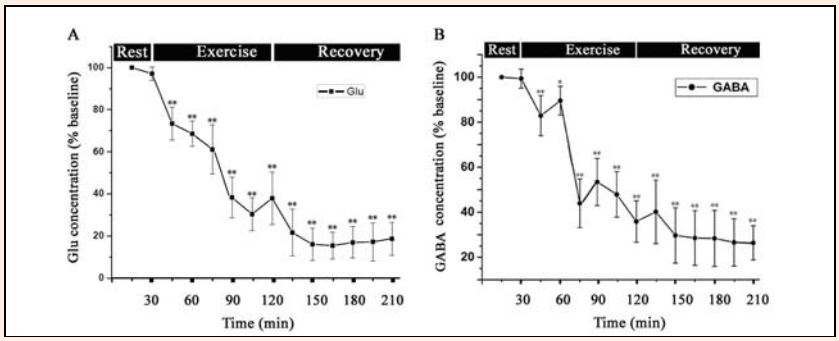

Figure 8. Dynamic changes of glutamic acid and gamma-aminobutyric acid levels in STN extracellular fluid with the development of fatigue. A, dynamic changes of glutamic acid. B, dynamic changes of gamma-aminobutyric acid. *p < 0.05, ** p < 0.01 *vs* rest condition.

(cortico-striato-GPi/SNr) and 'indirect' pathway (cortico-striato-GPe-STN-GPi/SNr) (Alexander and Crutcher, 1990). Two pathways have opposing effects on the basal ganglia output nuclei and are considered to be simultaneously involved in the control of voluntary movements (Nambu et al., 1997). Imbalance between the activities of the two pathways has been proposed to account for the hypo- and hyperkinetic features of basal ganglia related movement disorders (Chaudhuri and Behan, 2000). More recently, much emphasis has been placed on the 'hyperdirect' pathway (cortico-STN-GPi/SNr) since the critical role it plays in movement disorder disease, e.g., Parkinson disease (Nambu et al., 2000). STN receives direct excitatory glutamatergic afferent from frontal cortex and exerts an excitatory drive to GPi/SNr, and then exerts a strong inhibitive feedback to the motor cortex excitability through the relay of thalamus (Plenz and Kital, 1999). Thus, changes of STN activity plays critical role in modulating cortical activity and has been regarded as one of the most important nuclei of 'brake system' (Nambu et al., 2002). The modulatory effect of STN on cortex activity has been well documented, in parkinsonian animals (Périer et al., 2002) and patients (Alberts et al., 2008) with PD, STN activity is characterized with an augmented firing rate with bursting activity, however the activity of motor cortex decreased (Lefaucheur, 2005). Meanwhile, the patients are always characterized with some movement disorders such as bradykinesia, rigidity, tremor, and postural instability, fatigue is extremely common in PD patients as well (Friedman et al., 2011). In our experiment, the modulatory effect of STN on cortex activity was also found: behavioral study found that, rats adjusted themselves well to the preconditioned speed at the beginning of the exercise, about 45min later, the self-motivated exercise capacity decreased significantly. Correspondingly, electrophysiological character showed that significant increases were found in all slow bands (δ, θ band), α band and fast bands (β, γ band) waves, especially in fast band waves, of STN LFPs. Thus, the net changes of STN activity showed significant increase (f_g was significantly higher than that of the rest condition, $p < 0.05$). Meanwhile, the electrophysiological characters of ECoG altered in a reverse way: activity of slow band waves (δ band) increased and f_g decreased significantly ($p < 0.05$), which indicated the occurrence of fatigue (Arai et al., 2002). 45min after the onset of exercise, the increased STN activity, we presumed, exerted a strong depressive effect on cortical activity through STN-GPi/SNr-thalamus pathway, and thus induced the development of fatigue. This hypothesis was also supported by our previous work (Wang et al., 2010). In that study, the STN activity was suppressed through injecting the glutamate receptor antagonist, 6-methyl- 2-phenylethynyl-pyridine (MPEP), 10min before the start of the exhausting exercise, correspondingly, behavioral studies showed that, the time from the onset of the exercise to the emergence of the 'early fatigue' was significantly prolonged.

After the appearance of 'early fatigue', rats were not able to maintain the preconditioned speed without slight sound/light/electrical stimulation. Central fatigue is defined as the failure to initiate and/or sustain attentional tasks/physical activities requiring self motivation (as opposed to external stimulation) (Chaudhuri and Behan, 2000). We predict that the fatigue appeared 45min after the onset of exercise ('early fatigue') was CNS dominant fatigue (central fatigue). Then, rats continued their exercise with stimulation till exhaustion. Under exhaustion condition, f_g of the ECoG reached the minimum value, while, no significant changes of STN activity were found although activity of α and β bands significantly increased. We presumed that, the occurrence of fatigue at this moment was affected by both central and peripheral factors. This indicates that, the changes of STN activity contributes more to the development of 'early fatigue', mechanism underlying the subsequent decline of ECoG activity till exhaustion is much more complicated. It has been reported that, in human subjects the exercise-induced fatigue can be divided into central and peripheral fatigue, and the central fatigue precedes peripheral fatigue under some conditions (Rietjens et al., 2005; Kay et al., 2001), while, whether this two phase of fatigue ('early fatigue' and exhaustion) fund in animals in the present study is consistent with what fund in human subjects, further study is needed.

Pavlov's protective inhibition theory considered that, the activity of cortex might shift from excitatory condition to inhibitive condition gradually after longtime or high intensity tasks so as to avoid the further damage of neuron cell. In our study, activity of STN increased while cortex activity decreased with the appearance of 'early fatigue', this might be a critical approach for achieving the protective inhibition.

The modulating effect of Glu and GABA on STN activity during exercise

STN receives its major afferents from the cerebral cortex, thalamus, GPe and brainstem (Hamani et al., 2004). Glu, GABA, dopamine (DA) and 5-hydroxytryptamine (5-HT) were all detected within STN extracellular fluid (Bevan et al., 2000; Martinez-Price and Geyer, 2002; Shen and Johnson, 2000). Research reported that, Glu, DA and 5-HT play the excitatory effect while GABA exerts an inhibitive effect on STN activity (Flores et al., 1995). Glu and GABA play the dominant role in regulating STN activity (Ampe et al., 2007).

In our study, Glu and GABA levels in extracellular fluid all showed a decreasing trend. In rodent animals, Glu in STN extracellular fluid is partly transferred from the glutamatergic afferent projections, neurons in STN also synthetize Glu themselves and project Glu to GPe, GPi/SNr. With the proceeding of exercise, glutamatergic afferent projections from cortex released less Glu because of the declined activity of cortex; meanwhile, more Glu was released from STN to GPe/SNr following the increased activity of STN, that all account for the decreased level of Glu in STN. Otherwise, Glu is a non-essential amino acid and couldn't permeate through the blood brain barrier, so, all the Glu was generated from glucose and some other materials in the brain tissue. Previous study showed that, the concentration of glucose decreased with the development of fatigue during exhausting exercise (Yang et al., 2009). Therefore, the decrease of Glu in STN

following exhausting exercise may also be relevant to the decrease of glucose. GABA in STN extracellular fluid was mainly released through the GABAergic afferent from GPe. Previous study (Qiao et al., 2005) demonstrated that activity of striatum neuron increased significantly during exhausting exercise, we predicted that releasing of GABA from striatum as a consequence of its excitation might induce the decrease of GABA release from GPe to STN by inhibiting GPe activity through striatum-GPe-STN pathway.

Since Glu and GABA have opposite effect on STN activity, so, Glu/GABA is always used to reflect the neuron activity. In this study, 45min after the onset of exercise, Glu/GABA was significantly higher than that of the rest condition (Figure 9). Accordingly, behavioral study showed that the self motivated exercise capacity of the rats decreased obviously which implied the appearance of the 'early fatigue'. This result was consistent with the electrophysiological study. This indicated that, Glu/GABA was closely relevant to the development of 'early fatigue'. However, 15min after exhaustion, Glu/GABA was significantly lower than that of rest condition. This result was not consistent with the dynamic changes of STN activity observed by the electrophysiological study (Figure 7). Although, Glu and GABA are the dominant neurotransmitters in modulating STN activity, the effects of other neurotransmitters, such as DA and 5-HT, couldn't be ignored. And we presume that the differences described above might be relevant to the dynamic changes of other neurochemicals, and further study is needed.

Conclusion

In summary, the present study shows that, the development of the cortex fatigue during exhausting exercise consists of two phases, 'early fatigue' and exhaustion. Our results suggest that, dynamic changes of STN activity are closely relevant to the development of 'early fatigue' rather than exhaustion, and the changes of STN activity during the development of 'early fatigue' might be partly related to the variance of Glu and GABA levels in STN extracellular fluid.

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Key points

- The development of the cortex fatigue during exhausting exercise consists of two phases, 'early fatigue' and exhaustion.
- Dynamic changes of STN activity are closely relevant to the development of 'early fatigue' rather than exhaustion.
- The changes of STN activity during the development of 'early fatigue' might be partly related to the variance of Glu and GABA levels in STN extracellular fluid.

AUTHORS BIOGRAPHY

Dalei WANG
Employment
PhD student, College of Physical Education and Sports, Beijing Normal University, Beijing, 100875, China
Degree
MEd
Research interests
Exercise physiology.
E-mail: dlwang@iccas.ac.cn

Xiaoli LIU
Employment
Professor of College of Physical Education and Sports, Beijing Normal University, Beijing, 100875, China
Degree
PhD
Research interests
Exercise and nutritional sciences.
E-mail: xiaolil@bnu.edu.cn

Decai QIAO
Employment
Professor of College of Physical Education and Sports, Beijing Normal University, Beijing, 100875, China
Degree
PhD
Research interests
Effects of exercise on the physiological and biochemical functions of the brain and central nervous systems.
E-mail: decaiq@bnu.edu.cn

✉ **Decai Qiao**
College of Physical Education and Sports, Beijing Normal University, Beijing, 100875, China