EIGHT WEEKS OF MODERATE EXERCISE ATTENUATES IMPAIRMENT OF EMOTION AND COGNITION IN SLEEP DEPRIVATION RAT: A PILOT STUDY

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Abstract— Sleep deprivation is the condition whereby fail to have enough sleep. Several studies have indicated that sleep deprivation impairs emotional control and cognitive function through weakening prefrontal-limbic neural plasticity. The weakening plasticity is associated with functional impairment of neurotransmitter systems, uneven electrolyte distribution, and reversible blood-brain barrier dysfunction. Exercise, especially moderate-intensity exercise has been demonstrated to improve emotional control and cognitive function through increased neural activity in prefrontal-limbic system. Moreover, several reports indicated that exercise avoid the electrolyte imbalance as well as reduce the reversible disruption of blood-brain barrier integrity in prefrontal-limbic system. Although previous studies have extensively demonstrated the deleterious effects of sleep deprivation on mental function and the contrasting beneficial effects of exercise on this function. Nevertheless, there has thus far been relatively little research into the interaction of the two at the molecular level in prefrontal-limbic system. Based on the above-mentioned points, we hypothesize that exercise can reduce sleep deprivation-induced impairment of emotional control and cognitive function. The mechanism is mediated through activation of neurotransmitter systems, correction for the uneven distribution of electrolytes, and amelioration of blood-brain barrier dysfunction in prefrontal-limbic system. In our preliminary study, we have successfully constructed rat models of sleep deprivation and exercise to assess emotional control and cognitive function using behavioral tests and stress hormone monitoring. Our results showed that 8-week regular moderate exercise will attenuate impairment of emotion and cognition, but not the 4-week exercise. Future research will determine whether the affective behavioral responses with or without regular exercise following sleep deprivation are resulted from alteration of neurotransmitter reactivity, neurochemical expressions, and ionic changes in prefrontal-limbic system.

Index Terms— behavior, cognition, exercise training, sleep deprivatio.

I. INTRODUCTION

Background

Sleep deprivation is the condition whereby fail to have enough sleep. We are a society, people obtain insufficient sleep for many reasons, such as sleep disorders, psychiatric disturbance, or busy schedules (e.g. stay up all night to study, work, or have fun). Current estimates suggest that more than one-third of all adults receive less than 7 h of sleep per day (Perry et al., 2013). This troubling statistic is likely to remain unchanged or may become even worse in the near future, as many factors in our modern society prevent sufficient sleep. Sleep deprivation negatively impacts our mood, our ability to focus, and our ability to access higher-level cognitive functions (Orzel-Gryglewska, 2010; Killgore, 2012; Poudel et al., 2013). Furthermore, concentration, logical reasoning, mathematical capacity, and working memory are all aspects of cognitive function compromised by sleep deprivation (Walker, 2008; Diekelmann et al., 2009; Basner et al., 2013). However, not all of these functions rely on the same regions of the brain.

Traditionally, Neuroscientists have often described cognition and emotion as separable processes implemented by different regions of the brain, such as the prefrontal cortex for cognition and the limbic system for emotion (Wittmann and Wassenhove, 2009). In the recent year, many studies argued that consisting functional interactions between prefrontal cortex and limbic system mediate emotional influences on cognitive processes (Allen et al., 2011; Ray and Zald, 2012). Although several studies indicate that sleep deprivation impairs cognitive performance through weakening prefrontal-limbic neural plasticity (Havekes et al., 2012; Sil'kis et al., 2014). Nonetheless, there has thus far been relatively little research into the area.

Cognitive function involves the participation of many different neurotransmitter systems in a variety of brain areas, but mainly in prefrontal-limbic circuit (Wittmann and Wassenhove, 2009; Sil'kis, 2014). The centerpiece of investigation regarding cognitive function has classically been the cholinergic system, but it has become increasingly clear that the dopaminergic, and the serotonergic systems also provide the neural basis for cognitive function (Gurvich and Rossell, 2014; Seyedabadi et al., 2014). Increasing evidence suggest that chronic sleep deprivation-induced impairment on cognitive function concomitant with a disturbance of the above-mentioned transmitter systems (Chen et al., 2006; Vecsey et al., 2009; Tadavarty et al., 2011; Havekes et al., 2012). Profound studies demonstrated that maintenance of a blood-brain barrier and homeostasis of electrolyte in prefrontal-limbic areas associates with the cognitive functions (Oliveira and Bading, 2011; Saccardi et al., 2011; Gomez-Gonzalez et al., 2013; He et al., 2014; Peters et al., 2014). Interesting, our previous study has demonstrated that following sleep deprivation, calcium intensity is significantly decreased in prefrontal-limbic system (Chang et al., 2012).

Behavioral testing also showed poor responses after sleep deprivation. We suggest that impaired calcium expression will depress downstream prefrontal-limbic neuronal activation, which may contribute to the initiation or development of sleep deprivationrelated cognitive deficiency.

It is well known that exercise, especially moderate intensity exercise has numerous health benefits. For example, it contributes to more general improvement in brain health and repair (Podewils et al., 2005; Andel et al., 2008; Hötting and Röder, 2013; Bielak et al., 2014; Blondell et al., 2014). Substantial bodies of studies have revealed that exercise improves cognitive function via increased neural activity in prefrontal-limbic system (Baierlein et al., 2011; Birch et al., 2013; Cheng et al., 2013). Moreover, several reports indicated that exercise reduce the reversible disruption of blood-brain barrier integrity (Toborek et al., 2013; Zhang et al., 2013). Previous studies have extensively demonstrated the deleterious effects of sleep deprivation on cognitive function and the contrasting beneficial effects of exercise on this behavior. Nevertheless, there has thus far been relatively little research into the interaction of the two at the molecular level in prefrontal-limbic system.

Hypothesis

Exercise reduces sleep deprivation-induced impairment of emotional control and cognitive function is mediated through activation of neurotransmitter systems, correction for the uneven distribution of electrolytes, and amelioration of blood-brain barrier dysfunction in prefrontal-limbic system.

Objectives

This study is undertaken to provide a detailed account of the effect of exercise training on sleep deprivation-induced impairments on neurofunctional and neurostructural correlates in prefrontal-limbic system. The neural function is examined using behavioral tests. Cytochemistry, molecular imaging, and electron microscopy are used to investigate the changes of neurotransmitters levels, ion distributions, and blood-brain barrier permeability in prefrontal-limbic system, respectively.

The aim of the present study is to develop rat models of sleep deprivation and exercise to assess emotional control and cognitive function using behavioral tests and stress hormone monitoring to investigate if 4-week or 8-week regular moderate exercise will attenuate impairment of emotion and cognition.

II. MATERIALS AND METHODS

Ethics statement

The studies were carried out in accordance with the guide lines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. In addition, the protocol complies with the recommendations of Taipei Medical University and was approved by the Institutional Ethics Committee.

Animals

Male Wistar rats from our breeding colony were used, weighing 220–250 g at the beginning of the experiments. The animals were housed in groups of five, in polypropylene cages and maintained under standard conditions of temperature ($22 \pm 2 \circ C$) and illumination (12/12 h light–dark cycle). The animals had free access to water and food throughout the experiment.

Experimental models design

A summary of the experimental design is provided in Figure 1. The experimental groups are as follows:

1. Sedentary and sleep-deprivation group (Sed-SD(+) group, n = 5): animals remain sedentary and received sleep deprivation for 4 weeks.

2. Four weeks of exercise and sleep-deprivation group (4-wk Exe/SD(+) group, n = 7): animals are run on a treadmill for 4 weeks (from week 5 to week 8) before undergoing sleep deprivation treatment for 4 weeks.

3. Eight weeks of exercise and sleep-deprivation group (8-wk Exe/SD(+) group, n = 6): animals are run on a treadmill for 8 weeks (from week 1 to week 8) before undergoing sleep deprivation treatment for 4 weeks.
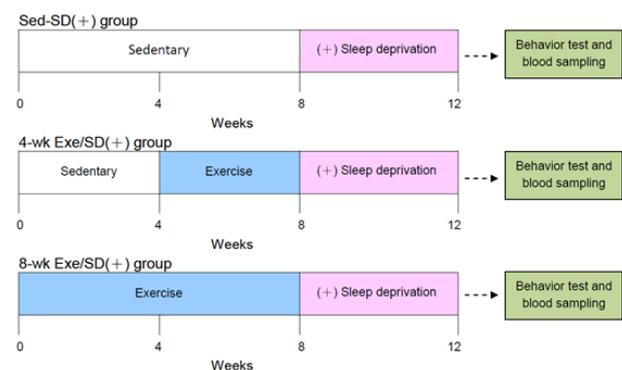

Fig. 1. Scheme of the experimental design

Treadmill exercise

Rats are familiarized with the treadmill apparatus to minimize novelty stress and are randomly assigned to different experimental groups (sedentary and exercised groups). Each rat ran on the treadmill at a low initial speed follows by an increase of 5 m/min in the speed of the treadmill every 3 min until they reach their point of exhaustion (i.e., failure of rats to continue running). The time to fatigue (in minutes) and workload (expressed by velocity in m/min) are taken as indices of exercise capacity, and as a measure of maximum oxygen uptake (VO2 max) (Arida et al., 1999; Cechetti et al., 2012). The moderate-intensity forced-exercise training program is used, which consists of running sessions on an adapted motorized rodent treadmill at 60% of each rat's maximal oxygen uptake (Brooks and White, 1978).

Animals in the exercise groups are trained on a treadmill (0° inclination) for 60 min/d, at a treadmill speed of 17 m/min, for 5 d/week. In order to encourage rats to run forward, the front end of the treadmill is covered with a dark cloth. Furthermore,

a shock grid at the back of treadmill provided a mild shock (0.75 mA, 500 ms duration,
0.5 Hz rate) to prod rats to run if the pace of the animals slow below the treadmill rate. Actually, very few shocks are applied in the first few minutes of the exercise and most animals run voluntarily without using shocks. Animals that are not able to perform the exercise are excluded from the sample. At the same time of the day, the sedentary rats are placed on a stationary treadmill, with the shock grid turn off, for the duration of the treadmill training session.

Sleep deprivation procedure and the electrophysiological recording

Sleep deprivation is performed by the disc-on-water method as described in our previous study (Chang et al., 2010; 2012). This method is chosen because it has previously been validated as able to produce effective sleep deprivation in one animal without excessive physical exertion (Bergmann et al., 1989). Briefly, the apparatus comprised two rectangular clear plastic chambers (60 x 20 x 60 cm each) places side by side. A single plastic disc (40 cm in diameter), which can be rotated by a computerized monitoring system, serving as the rat-carrying platform is built into the lower quarter of the two chambers. Beneath the disc, and extending to the chamber walls, is a rectangular tray filled with water to a depth of 5 cm. Before the experiment began, a rat to be sleep-deprived and its yoked control are placed in the total sleep deprivation apparatus for at least 7 days for environmental adaptation.

During this period, the chambers are fitted with a solid mat in place of the water. Sleep deprivation depends on the rats' aversion to water, as rats rarely enter water spontaneously. When sleep onset is detect in the sleep deprived rat, the disk is rotated slowly by the computerized monitoring system at a moderate speed of 3.5 rpm, forcing both rats to keep awake and walk against the direction of disc rotation to avoid being forced into the water. When the sleep deprived rat is spontaneously awake, the disc is stationary.

Total sleep deprivation is performed consecutively for 5 days, and follow by 2 days of rebound. The total sleep deprivation-rebound cycle is constantly repeated for 4 times in which the total period of sleep deprivation is persisted for 4 weeks. During the adaptive period and the whole time of experiment, all animals are exposed to an automatically regulated light-dark cycle of 12:12 h at a constant temperature of $23 \pm 1°C$ and a relative humidity of 60 %. Food and water are made available through grids placed on top of the chambers. In the care and handling of all experimental animals, we follow the Guide for the Care and Use of Laboratory Animals (1985) as stated in the United States NIH guidelines (NIH publication no. 86-23).

All the sleep deprivation procedures are also approved by the Laboratory Animal Center Authorities of the Taipei Medical University. Electroencephalographic and electromyographic data are recorded on a Grass model 78 polygraph (Grass-Telefactor, West Warwick, RI, USA) and relayed to a computer for digital recording. Data are divided into 30-s epochs and scored as waking, non-rapid eye movement, or rapid eye movement sleep using an automated

scoring system previously validated against visual and behavioral methods (Bergmann et al., 1989).

Forced-Swim Test

Rats are placed individually in a tank (50 cm height, 30 cm diameter) filled with water to a depth of 25 cm, at 251C, for 15 min. After each test, rats were dried with a towel and placed in a separate cage, heated by a 300W lamp until the animals were dry. Rats were videotaped during the test for further scoring. Behavioral scoring was performed as described by Detke et al (1995). Immobility was defined as the behavior consisting of floating or movement of hind limbs directed exclusively to maintain the head off the water. Climbing was defined as forceful attempts to climb the wall of the tank with either forepaws, or trying to jump out of the tank. Swimming was defined as an active behavior, other than movements characterized as climbing, and which involved displacement around the surface of the water. The behavioral test was scored by a single observer, blind to the treatment condition. Quantification of behaviors was performed by recording the predominant behavior in each 5 s period (Cryan and Lucki, 2000).

Plasma corticosterone assays

The blood samples used for plasma corticosteroneis collected from the left ventricle during the transcardiac perfusion. The blood is first kept in the K2E EDTA K2 tubes placing on ice and then centrifuged at 2600 g at 4 °C for 15 min. The supernatant is amassed and both plasma corticosterone and ACTH levels are determined by the radioimmunoassay method (corticosterone kit, MP Biomedicals, Costa Mesa, USA; ACTH kit, Nichols Institute Diagnostics, Bad Vilbel, Germany).

Statistical analysis

Data are analyzed using SPSS (version 19.0). After confirmation of the normality of variables using the Shapiro–Wilk test, the values are compared using a one-factor analysis of variance (ANOVA). All analyses of variances were followed by the Bonferroni correction for multiple comparisons, whenever appropriate. Differences with $p < 0.05$ were considered statistically significant.

III. RESULTS AND DISCUSSION

Table 1. Activity in the forced-swim test induced by 4-wk sleep deprivation (second, Mean ± SD).

	Immobility	Swimming	Climbing
Sed-SD	143.1 ± 8.3	45.3 ± 4.1	57.9 ± 10.5
4-wk Exe/SD	107.9 ± 9.5*	101.8 ± 13.2*	44.1 ± 7.3
8-wk Exe/SD	96.7 ± 11.4*	95.1 ± 11.4*	52.5 ± 7.0

* Statistically significant difference when care to the sedentary group. Sed-SD group, sedentary and sleep-deprivation group animals; 4-wk Exe/SD group, four weeks of exercise and sleep-deprivation group; 8-wk Exe/SD group, eight weeks of exercise and sleep-deprivation group

Both 4-wk and 8-wk exercise group are less immobility than sedentary group ($p < 0.01$ and 0.005, respectively). Exercised rats swam more than sedentary rats (both $ps < 0.01$). Climbing behavior did not differ among groups (Table 1).

Table 2 shows 8-wk exercise group has lower corticosterone concentration than 4-wk exercise group and sedentary group (p = 0.017 and 0.011, respectively).

Table 2. Blood stress hormone concentration after 4-wk sleep deprivation (Mean ± SD).

	Corticosterone (ng/mL)
Sed-SD group	568 ± 87
4-wk Exe/SD group	538 ± 55
8-wk Exe/SD group	371 ± 56*

* Statistically significant difference when care to the sedentary group. Sed-SD group, sedentary and sleep-deprivation group animals; 4-wk Exe/SD group, four weeks of exercise and sleep-deprivation group; 8-wk Exe/SD group, eight weeks of exercise and sleep-deprivation group.

In our preliminary study, we have successfully constructed rat models of sleep deprivation and exercise to assess emotional control and cognitive function using behavioral tests and stress hormone monitoring. Our results showed that 8-week regular moderate exercise will attenuate impairment of emotion and cognition in sleep-deprivation rat, but not the 4-week exercise.

Future research will determine whether the affective behavioral responses with or without regular exercise following sleep deprivation are resulted from alteration of neurotransmitter reactivity, neurochemical expressions, and ionic changes in prefrontal-limbic system.

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