Effect of aging on treadmill exercise induced theta power in the rat

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Abstract The effects of aging on the electroencephalogram (EEG) power spectra of 8- and 60-week-old Wistar–Kyoto rats were examined during the waking baseline and treadmill exercise. Using continuous and simultaneous recordings of EEG and electromyogram signals, this study demonstrated that the alpha (10–13 Hz), theta (6–10 Hz), and delta (0.5–4 Hz) powers of the EEG were significantly lower in older rats as compared with young rats during the waking baseline. In the young rats, treadmill exercise resulted promptly in a higher alpha power, higher theta power, and higher theta power percentage as compared with the waking baseline. In the aged rats,

treadmill exercise only resulted in a higher theta power and higher theta power percentage. During the treadmill exercise, however, the aged rats still showed a significantly lower exercise-evoked theta power change than the young rats. These results suggested that aging is accompanied by lower EEG activities during waking and this also is accompanied by an attenuated response of the brain to exercise in the rat.

Keywords EEG spectra · Waking status · Treadmill exercise · Aging · During exercise · Theta power of the EEG

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Introduction

Many brain functions, including the response to stimulation, sleep quality, memory, and cognitive function, are known to be deficient in older animals (Bach et al. 1999; Blanco-Centurion and Shiromani 2006; Buchanan and Powell 1988; Orr et al. 2001; Solomon and Groccia-Ellison 1996; St Jacques and Levine 2007; Tombaugh et al. 2002; van Praag et al. 2005) and humans (Backhaus et al. 2007; Benloucif et al. 2004; Cummins and Finnigan 2007; Hess 2005; Prinz 1995). Exercise, regular physical activity, and good physical fitness are widely accepted as factors that improve a number of health outcomes (Cotman and Berchtold 2002; Ide and Secher 2000; Kramer et al. 2005; Li et al. 2008; Myers et al. 2002; Thompson et al. 2003) and promote memory and other cognitive functions (Colcombe and Kramer 2003; Hillman et al. 2002; Kramer et al. 1999, 2005; Li et al. 2008; Sim et al. 2004). Furthermore, the beneficial effects of exercise intervention on memory and other aspects of cognition have been documented for elderly persons (Benloucif et al. 2004; Dustman et al. 1984; Laurin et al. 2001; McMurdo and Burnett 1992; Molloy et al. 1988; Okumiyama et al. 1996; Perrig-Chiello et al. 1998; Rogers et al. 1990; Stones and Dawe 1993; Williams and Lord 1997) and for rats (Blanco-Centurion and Shiromani 2006; van Praag et al. 2005). However, the underlying electrophysiological mechanisms associated with exercise-related memory and cognitive function changes are still unclear.

The theta power of the electroencephalogram (EEG) is usually far more prominent during active waking (Nerad and Bilkey 2005). Evidence from humans (Cummins and Finnigan 2007; Doppelmayr et al. 2000; Klimesch 1999; Laukka et al. 1995) and rats (Landfield et al. 1972; Olvera-Cortes et al. 2004; Orr et al. 2001) has suggested that the theta power of the EEG represents some special cognitive functions and/or special learning-related functions. An increase in theta power while awake may be related to higher cognitive functions and special learning functions (Cummins and Finnigan 2007; Klimesch 1999; Landfield et al. 1972; Laukka et al. 1995; Olvera-Cortes et al. 2004). Some animal studies have revealed that free running may evoke the theta power of the EEG (Angyan and Czopf 1998; Buzsaki et al. 1981; Crabbe and Dishman 2004; Gengler et al. 2005; Nielsen et al. 2001; Slawinska and Kasicki 1998;

Teitelbaum et al. 1975); however, free running is not equivalent to exercise. Whether controlled (treadmill) exercise may evoke theta power of the EEG has rarely been reported on. In addition to theta power, there are other rhythmic components of the EEG such as alpha power, beta power, and delta power. Alpha power and beta power are also related to the waking state (Makeig and Jung 1995; Nerad and Bilkey 2005). Delta power, in contrast, is related to sleep and is usually used to indicate the depth of sleep (Yang et al. 2003). Recently, we establish a free moving rat model that can be used to study various cerebral mechanisms simultaneously during treadmill exercise; this offers the possibility of collecting electrophysiological data during exercise (Li et al. 2008). We found that treadmill exercise resulted promptly in a higher theta power in young rats. Such changes were quickly reversed when the treadmill was stopped. In this context, we ask whether exercise-evoked theta power shows significant age-related changes, which may parallel previously reported memory and cognitive alterations.

It is well known that rats undergo frequent changes in their sleep/wake state while free moving (Borbely 1976; Kuo et al. 2004b; Trachsel et al. 1991; Yang et al. 2003). Without classifying the sleep/wake status, EEG spectra powers will dramatically fluctuate over time and delicate results may be masked. By classifying the sleep/wake status by the EEG and electromyogram (EMG) signals, the present study was designed (1) to confirm whether brains of awake young rats showed a significant response to treadmill exercise when compared to before exercise, (2) to determine whether older rats have a lower baseline level and/or show a lower response to exercise than young rats, and (3) to determine whether treadmill exercise is still able to evoke brain activity in the older rats.

Materials and methods

Preparation of animals

The experiments were carried out on 8-week-old (young, $n=8$) and 60-week-old (middle-aged, $n=8$), male Wistar-Kyoto rats. The rats were raised in a sound-attenuated room under a 12:12-h light–dark cycle (08:00–20:00 lights on) at a controlled temperature ($22\pm2^\circ\text{C}$) and humidity (40–70%). The detailed procedures of the surgery have been described

previously (Kuo et al. 2004a, b; Shaw et al. 2002). On the day of electrode implantation, the young rats were 7 weeks old and the middle-aged rats were 59 weeks old. Under pentobarbital anesthesia (50 mg/kg, i.p.), each rat was placed in a standard stereotaxic apparatus, and then the electrodes for the occipital EEG and nuchal EMG were implanted. The dorsal surface of the skull was exposed and cleaned. Two stainless steel screws were driven bilaterally into the skull overlaying the occipital (4.0 mm posterior to and 2.0 mm lateral to the bregma) regions of the cortex. A reference electrode was implanted 2.0 mm caudal to the lambda. The electrodes did not penetrate the underlying dura. Two seven-strand stainless steel microwires were bilaterally inserted into the dorsal neck muscles to record EMG. After surgery, the rats were treated with an antibiotic (chlortetracycline) and individually housed in translucent cages for 1 week of recovery.

When recovered, the rats were subjected to the experimental regime as follows. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. Animals were familiarized with the motorized treadmill (Model T510E, Diagnostic & Research Instruments Co., Taoyuan, Taiwan) by running on the treadmill for 10 min on three consecutive days (belt speed=8 m/min). All the signals were relayed to a recorder by wires through a common connector that was fixed on the head (Shaw et al. 2002). The bioelectrical signals were recorded using a miniature physiological data recorder (KY1C, K&Y Lab, Taiwan). During the recording day, electrophysiological signals were recorded for 80 min (06:00–07:20) during the dark period on the treadmill. The first 20-min period was allowed for the rat to become familiar with its surroundings. Then, 30 min of moderate exercise (13 m/min) was performed by each rat and followed by 30-min recovery. Rats were observed while exercising so we can ensure that they were willing to run continuously.

Data acquisition and storage

Data acquisition and storage were similar to the procedure described previously for sleep (Kuo et al. 2004a, b; Shaw et al. 2002) and running research (Li et al. 2008). The EEG and EMG signals were amplified 1,000-fold. The EEG was filtered at 0.16–

48 Hz and the EMG at 34–103 Hz. The EEG and EMG signals were synchronously digitized using an analog-digital converter with different sampling rates (125 and 500 Hz, respectively). The acquired dataset was then analyzed on-line but was simultaneously stored on a flash memory for subsequent off-line analysis.

Digital signal processing and power spectral analysis

The digital signal processing of the bioelectric signals was similar to the procedures used in our previous studies (Kuo et al. 2004a, b). The sampling rate of the EEG signals was reduced to 64 Hz. The EEG signal to be analyzed was truncated into successive 16-s (1,024 points) time segments (windows or epochs) with a 50% overlap. A Hamming window was applied to each time segment to attenuate the leakage effect (Kuo and Chan 1993). Our algorithm then estimated the power density of the spectral components based on fast Fourier transformation (FFT). The resulting power spectrum was corrected for attenuation resulting from the sampling and the application of the Hamming window. The EMG signals to be analyzed were truncated into successive 2-s (2,048 points) time segments without overlapping and then they underwent FFT after application of the Hamming window. Eight successive EMG spectra (total of 16 s) were averaged to achieve synchronization with the EEG spectra.

For each 16-s time segment, we quantified the total power (TP, 0.5–32 Hz), delta power (0.5–4 Hz), theta power (6–10 Hz), alpha power (10–13 Hz), and beta power (13–32 Hz) of the EEG spectrogram (Nerad and Bilkey 2005) as well as the power of the EMG spectrogram. We also calculated the mean power frequency (MPF) of the EEG spectrogram, which covered all these components using the following equation (Kuo et al. 2004a, b):

$$\text{MPF} = \frac{\sum_{f=f_0}^{f_c} f \times \text{PSD}(f)}{\sum_{f=f_0}^{f_c} \text{PSD}(f)}$$

where f is any given frequency, f_0 is the lower cutoff frequency, f_c is the upper cutoff frequency, and $\text{PSD}(f)$ is the power spectral density of a given frequency. In this study, the f_0 was the low-cut frequency of the delta power whereas the f_c was the high-cut frequency of the beta power.

Sleep analysis

Sleep analysis was according to our previously developed and semi-automatic computer procedure, which has been described in detail elsewhere (Kuo et al. 2004a, b, 2008; Kuo and Yang 2005). The procedure discriminates the consciousness states into active waking (AW), quiet sleep (QS), and paradoxical sleep (PS). Briefly, continuous power spectral analysis was applied to the EEG and EMG signals, from which the MPF of the EEG and the power magnitude of the EMG were quantified. The duration of the time segments was 16 s and successive time segments were 50% overlapped. Thus, the time resolution of the sleep scoring was 8 s. For each time segment, the sleep/wake stage was defined as AW if the corresponding MPF was greater than a pre-defined MPF threshold (TMPF) and the EMG power was greater than a pre-defined EMG power threshold (TEMG), as QS if the corresponding MPF was less than the TMPF and the EMG power was less than the TEMG, and as PS if the corresponding MPF was greater than the TMPF and the EMG power was less than the TEMG.

Statistical analysis

The distributions of the total, alpha, beta, theta, and delta powers of the EEG spectrogram and the power of the EMG spectrogram were not normal but were skewed to the right; therefore, they were logarithmically transformed to correct the distribution skewness. The power percentages of the EEG spectrogram were calculated from the specific powers divided by total power of the EEG spectrogram. Effects of the exercise (before and during exercise) and animal groups (the young and middle-aged groups) on the physiological parameters were assessed using two-way analysis of variance with repeated measures. When indicated by a significant *F* statistic, the regional differences were isolated using post hoc comparisons by the Fisher's least significant difference test. Comparisons between two sets of data were carried out by Student's *t* test. Statistical significance was assumed for $p < 0.05$. Values are expressed as mean $\pm$ SEM. Differences between values and 0 were performed with a 95% confidence interval analysis.

Results

Figure 1 represents a typical example of the changes in the EEG spectra before, during, and after treadmill exercise at 13 m/min in one young rat and one middle-aged rat. Compared with the young rat, the lower EEG spectral power of the middle-aged rat during all of the recording period is obvious. Compared with before exercise, the theta power of the EEG in both rats stood out during the entire exercise period. This phenomenon was reversed when the treadmill exercise stopped.

During active waking baseline: older rats had a lower EEG spectra and a higher beta power percentage of the EEG

In order to conserve the higher temper resolution and exclude the effect of sleep, we applied two types of analysis: (1) without classifying the sleep/wake status with a 2-min epoch temporal resolution and (2) classification of the sleep/wake status with a 5-min epoch temporal resolution. With the 2-min epoch temporal resolution, we found that middle-aged rats had a lower total power, theta power, and for some time points, delta power; in addition, for some time points of alpha and beta power percentage among middle-aged rats was higher than young rats during baseline (Figs. 2a, 3a, and 4a). After classifying sleep/wake status with a 5-min epoch temporal resolution, quantitative analyses additionally revealed that middle-aged rats had lower total, alpha, theta, and delta power and higher beta power percentage of the EEG than young rats during awake state baseline (Figs. 2b, 3b, and 4b).

During exercise: exercise may reverse some aged-related decline of the EEG powers

In order to manifest the EEG spectra and the EEG power percentage affected by exercise, the EEG spectra differences between the baseline, especially during active waking status, and exercise were further calculated and analyzed. Compared with the baseline (last time point, we confirmed that at this point, for each rats, it was awake) with a 2-min epoch temporal resolution, treadmill exercise resulted promptly in a higher MPF, higher alpha, beta, and theta power of the EEG and a higher theta

Fig. 1 Continuous and simultaneous analysis of the electroencephalogram (EEG) and electromyogram (EMG) before, during (20–50 min, belt speed=13 m/min), and after running on a treadmill for one young rat and for one middle-aged rat. Power spectrograms of the EEG (EPSD) show successive changes in power spectral density. Temporal alterations in total power (TP), beta power, alpha power, theta power, and delta power as well as mean power frequency (MPF) of the EEG are also shown. Ranges of frequencies on the beta, alpha, theta, and delta powers are denoted on the right side of the spectrograms. *ln* natural logarithm

power percentage of the EEG, but some points of the delta power and delta power percentage of the EEG were lower in the young rats. Such changes were quickly reversed when the treadmill stopped (Figs. 2a, 3a, and 4a). Like the young rats, the middle-aged rats also had a prominently higher theta power and theta power percentage as well as a lower delta power percentage during exercise. However, the other parameters of the middle-aged rats did not respond (Figs. 2a, 3a, and 4a). Furthermore, the middle-aged rats had a significantly less exercise-evoked theta power change than young rats (Figs. 3a and 4a). The differences between the middle-aged and young rats for some EEG spectra (alpha, beta, and theta powers) became noteworthy during exercise (Fig. 3a).

There were some different responses after classifying the sleep/wake status with 5-min epoch temporal resolution (Figs. 2b, 3b, and 4b). Compared with the waking baseline before exercise, treadmill exercise resulted promptly in a higher MPF, alpha, and

theta powers of the EEG and a higher theta but lower delta power percentage of the EEG in young rats. However, in contrast to these changes, exercise in middle-aged rats evoked the EEG changes that were all insignificant. In addition, when we considered the differences in the EEG spectral components for alpha power, beta power, and theta power, the theta and delta power percentage difference between young and middle-aged rats was even more noteworthy during exercise than before and during recovery (Figs. 3b and 4b). The differences between middle-aged and young rats for total power, delta power, and beta power percentage of the EEG disappeared during exercise.

Middle-aged rats had less exercise-evoked theta power changes

In order to manifest the component of theta power frequency and amplitude affected by the different recording systems, the peak frequency and amplitude

Fig. 2 Changes in total power (TP) and mean power frequency (MPF) of the electroencephalogram and electromyogram power (EMG) for different time points including before, during (20–50 min, oblique line, belt speed=13 m/min), and after running on the treadmill in young and middle-aged rats without classifying the sleep/wake status with a 2-min epoch time resolution (a). Changes in TP, MPF, and EMG for the different states including before (Con), during (0–10 min, E1; 20–30 min, E2), and after running (0–10 min, R1; 20–30 min, R2)

of theta power during PS between young and middle-aged rats were compared (Fig. 5a, b). The results showed no significant difference, which suggests that we can exclude any effect caused by the different recording systems. In order to clarify the frequency and amplitude changes of exercise-evoked theta power, we further compared the differences and changes between middle-aged and young rats for the active waking baseline and during exercise. The peak frequency and amplitude of theta power from exercise was subtracted from active waking baseline in the same rat to offset the effects caused by variation between the rats (Fig. 5c, d). Our results revealed that

on the treadmill in young and middle-aged rats after classifying the sleep/wake status with a 5-min epoch time resolution (b). Data are expressed as mean $\pm$ SEM of eight rats/group. * $p < 0.05$ vs. the same time point of the young rats. † $p < 0.05$ vs. the last point before exercise or the Con of the same group. ‡ $p < 0.05$ vs. the last point during exercise or the E2 of the same group. $\ln$ natural logarithm. The last points before and during exercise were under the waking status

middle-aged rats had a similar theta peak power frequency but had a significant lower theta power than young rats during waking baseline (Figs. 3, 4, and 5a, b). Exercise significantly increased the theta peak power frequency by about 0.3 Hz (Fig. 5a, c) and also significantly evoked theta power by about 1 $\ln(\mu V^2)$ (Fig. 5b, d) in young rats. Exercise had no such effect on middle-aged rats in terms of theta peak power frequency but did evoke a significant increase in theta power of about 0.7 $\ln(\mu V^2)$ (Fig. 5a–d). Nonetheless, overall, middle-aged rats had a significantly less exercise-evoked theta power change than young rats (Fig. 5).

Fig. 3 Changes in alpha, beta, theta, and delta power of the electroencephalogram (EEG) for different time points including before, during (20–50 min, oblique line, belt speed=13 m/min), and after running on the treadmill in young and middle-aged rats without classifying the sleep/wake status with a 2-min epoch time resolution (**a**). Changes in alpha, beta, theta, and delta power of the EEG for different states including before (Con), during (0–10 min, E1; 20–30 min, E2), and after running (0–10 min, R1; 20–30 min, R2) on the treadmill in young and middle-aged rats after classifying the sleep/wake status with a 5-min epoch time resolution (**b**). Data are expressed as mean $\pm$ SEM of eight rats/group. * $p < 0.05$ vs. the same time point of the young rats. † $p < 0.05$ vs. the last point before exercise or the Con of the same group. ‡ $p < 0.05$ vs. the last point during exercise or the E2 of the same group. $\ln$ natural logarithm. The last points before and during exercise were under the waking status

Discussion

With the capability of simultaneously detecting EEG and EMG during running, our experimental model was able to observe the effect of aging on brain activities in rats not only while awake but also during exercise. The results of the study are compatible with those that have been reported in humans and for rats using the special task test (Benloucif et al. 2004; Blanco-Centurion and Shiromani 2006; Colcombe and Kramer 2003; Dustman et al. 1984; Hillman et al. 2002; Kramer et al. 1999, 2005; McMurdo and Burnett 1992; Molloy et al. 1988; Okumiyama et al. 1996; Perrig-Chiello et al. 1998; Rogers et al. 1990;

Sim et al. 2004; Stones and Dawe 1993; van Praag et al. 2005; Williams and Lord 1997). We have demonstrated that aging in rats may lower theta power EEG spectra during waking and produce a lower response to running stimuli. Overall, we offered electrophysiological evidence for aging-related changes in exercise-evoked theta power.

Many studies have demonstrated that theta power has event-related synchronization with working memory tasks both in humans (Cummins and Finnigan 2007; Doppelmayr et al. 2000; Klimesch 1999; Laukka et al. 1995) and in animals (Landfield et al. 1972; Olvera-Cortes et al. 2004; Orr et al. 2001). Other studies have demonstrated that human theta

Fig. 4 Changes in alpha, beta, theta, and delta power percentage of the electroencephalogram (EEG) for different time points including before, during (20–50 min, oblique line, belt speed=13 m/min), and after running on the treadmill for young and middle-aged rats without classifying the sleep/wake status with a 2-min epoch time resolution (a). Changes in alpha, beta, theta, and delta power percentage of the EEG for different states including before (Con), during (0–10 min, E1; 20–30 min, E2), and after running (0–10 min, R1; 20–30 min, R2) on the treadmill in young and middle-aged rats after classifying the sleep/wake status with a 5-min epoch time resolution (b). Data are expressed as mean $\pm$ SEM of eight rats/group. * $p < 0.05$ vs. the same time point of the young rats. † $p < 0.05$ vs. the last point before exercise or the Con of the same group. ‡ $p < 0.05$ vs. the last point during exercise or the E2 of the same group. *ln* natural logarithm. The last points before and during exercise were under the waking status

oscillations are related to sensorimotor integration and special learning (Caplan et al. 2003). In addition, decreased theta event-related synchronization during working memory activation has been shown to be associated with progressive mild cognitive impairment (Missonnier et al. 2006). It is accepted that the increase in theta power observed during the awake state may relate to higher cognitive functions and special learning functions (Cummins and Finnigan 2007; Klimesch 1999; Landfield et al. 1972; Laukka et al. 1995; Olvera-Cortes et al. 2004). Rather than use behavior-based tasks, the present study explored evoked theta oscillations created during treadmill

exercise. Studies have suggested that exercise may affect various aspects of the body's physiological functioning (Cotman and Berchtold 2002; Ide and Secher 2000; Kramer et al. 2005). Regular physical activity and good physical fitness are widely accepted as factors that improve various health outcomes (Davy et al. 1998; Ekelund et al. 1988; Goldsmith et al. 1992; Myers et al. 2002; Thompson et al. 2003) and promote memory and cognitive functioning (Colcombe and Kramer 2003; Hillman et al. 2002; Kramer et al. 1999, 2005; Sim et al. 2004). Having overcome technical difficulties, our previous study successively described simultaneous changes in cere-

Fig. 5 Changes in theta peak power frequency (a) and theta power amplitude (b) of the electroencephalogram (EEG) for different states including paradoxical sleep (PS), waking baseline (Con), and treadmill exercise (Ex, belt speed=13 m/min) in young and middle-aged rats. The changes in theta peak power frequency (c) and in theta power amplitude (d) of the

EEG between treadmill exercise and waking baseline are also shown. Data are expressed as mean $\pm$ SEM of eight rats/group. * $p < 0.05$ vs. young rats by two-way ANOVA or t test. [†] $p < 0.05$ vs. waking baseline (Con) of the same group. [‡] $p < 0.05$ vs. 0 by 95% confidence. $\ln$ natural logarithm

bral activity during exercise in rats. We found that the EEG theta power and the EEG theta power percentage promptly increase during exercise (Li et al. 2008). This study expands this by further demonstrating that such effects are dependent on age (Fig. 5).

Previous animal studies have demonstrated that free running may evoke the EEG theta power and this has been related to the speed of locomotion and does not seem to be affected by aging (Shen et al. 1997; Slawinska and Kasicki 1998). However, free running is not equivalent to exercise. Whether controlled (treadmill) exercise may evoke theta power of the EEG really has not been explored. In this study, we controlled the speed of running in both groups and it was found that theta power induced by exercise was significantly affected by aging. The treadmill exercise model also controlled the experimental environment and provided fixed and limited spatial information, which has been reported to influence EEG theta waves through the spatial cognition pathway (Cummins and Finnigan 2007; Klimesch 1999; Landfield et al. 1972; Olvera-Cortes et al. 2004). The selected exercise strength (13 m/min for 30 min) was moderate and accepted by both young and older rats. The EMG

activity exhibited no differences between the two groups (Fig. 2), supporting the idea that the exercise was well controlled. A detailed comparison of free running and treadmill running under the same exercise strength is expected to provide further important information about the interaction of exercise and cognition.

Unlike humans, rats sleep both during the day and during the night with frequently sleep/wake changes when free moving (Borbely 1976; Kuo et al. 2004a, b; Trachsel et al. 1991). It is hard to discriminate the sleep/wake state of such rats without recording and analyzing the EEG and EMG signals simultaneously. Furthermore, EEG activities are known to be very different during these two states. Without discriminating the sleep/wake state, especially during the baseline and recovery periods, the sleep or waking transitions themselves may produce larger fluctuations than the test stimulation such as exercise (Fig. 1). Recently, we developed a simple but effective method to record EEG and EMG of free moving rats simultaneously in order to assess their sleep/wake status (Kuo et al. 2004a, b) and such measurement are even possible during treadmill exercise (Li et al. 2008). By classifying sleep/wake

status of the rats using their EEG and EMG signals, the present study demonstrated that there were significant EEG spectral differences between middle-aged and young rats that were awake when baseline and exercise were compared. Firstly, as others have found, elderly subjects have relative lower theta and delta powers and a lower absolute alpha power of their EEG (Cummins and Finnigan 2007; Klimesch 1999; Laurin et al. 2001; Mann and Roschke 2004; Naylor et al. 1998; Prinz 1995; Shiromani et al. 2000). In this study, we have additionally showed that, among middle-aged rats, there is a higher EEG beta power percentage compared to young rats during waking baseline (Figs. 2b, 3b, and 4b). Secondly, the present study reveals that exercise-evoked theta power changes occur, including frequency and amplitude, and that these are significantly less for the middle-aged rats than the young rats (Fig. 5). Thirdly, the differences in alpha power, beta power, theta power, theta power percentage, and delta power percentage of the EEG spectra components when young and middle-aged rats were compared are even greater during exercise than at baseline and during recovery (Figs. 3 and 4). The values for the EEG theta power and the EEG theta power percentage of middle-aged rats during treadmill exercise are reversed and close to the values obtained for young rats during the waking baseline (Figs. 3 and 4). However, the differences in total power, delta power, and beta power percentage between middle-aged and young rats during baseline disappeared during exercise (Figs. 2, 3, and 4). Since our experiments were carried out near the beginning of the light-on period of the rats' day, one question that can be raised is whether it is possible that the findings are dependent on the consciousness or sleepiness of these rats. The MPF, which has been broadly used to quantify the arousal state (Kuo et al. 2004b; Luoh et al. 1994), exhibits no significant difference between the young and older rats during the experimental period (Fig. 2). Thus, the results cannot be explained by the difference in the rats' arousal state.

The spectra components of the EEG have been categorized into four components according to their frequency range. The alpha power, beta power, and theta power of the EEG are dominant during the waking state (Nerad and Bilkey 2005). The increase in brain activity indicated by the alpha and beta powers of the EEG may be associated with the vigilance, alertness, and

attention (Makeig and Jung 1995). Middle-aged rats had lower overall total power, alpha power, theta power, and delta power and a higher overall beta power percentage of the EEG. This may represent the fact that middle-aged rats show less attention but have higher alertness and vigilance during the waking state before exercise. Delta power has been used to indicate the depth of sleep (Yang et al. 2003). It is noteworthy that our study has demonstrated that the delta power percentage drops more significantly in young rats than in middle-aged rats during exercise. These findings may indicate an aging-related deficit response to exercise associated with a loss of sleepiness during the treadmill exercise.

In the rat, theta power is usually far more prominent during active waking and PS (Blanco-Centurion and Shiromani 2006; Nerad and Bilkey 2005). However, few if any studies have described these types of changes in brain activity during exercise. This study provided an example of the whole process starting from before exercise and including both during exercise and also recovery after exercise. The weaker theta power in the middle-aged rats was partially and temporarily reversed during the treadmill exercise although it is still significantly weaker than that of the exercising young rat. The increase in theta power and MPF during running may indicate an alertness or attention state. The underlying mechanisms causing the above are of interest. For example, this study used a constant speed (13 m/min) for the treadmill exercise of both young and middle-aged rats. Although this speed is considered to be moderate exercise for a young animal, this might be different for older rats since physical fitness decreases with advancing aging. The nuchal EMG data (Fig. 2), however, did not exhibit any significant difference between the young and older rats during exercise. Although preliminary, there is no evidence at present time that indicates a difference in perceived exertion between the two groups. The long-term effects of exercise training as well as whether the changes in EEG are related to memory and other cognitive functions warrant further exploration.

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