Voluntary and Involuntary Running in the Rat Show Different Patterns of Theta Rhythm, Physical Activity and Heart Rate

Jia-Yi Li $^{1,2,4}$, Terry B. J. Kuo $^{1,2,3,4}$, Jiin-Cherng Yen $^5$, Shih-Chih Tsai $^4$, Cheryl C. H. Yang $^{1,2,3,4}$.

$^1$Institute of Brain Science, National Yang-Ming University, Taipei 11221, Taiwan;
$^2$Sleep Research Center, National Yang-Ming University, Taipei 11221, Taiwan;
$^3$Brain Research Center, National Yang-Ming University, Taipei 11221, Taiwan;
$^4$Department of Education and Research, Taipei City Hospital, Taipei 10341, Taiwan;
$^5$Institute of Pharmacology, National Yang-Ming University, Taipei 11221, Taiwan

The total number of pages: 29  The total number of figures: 6;  The total number of words: 7758

Grant sponsor: Ministry of Education, Aim for the Top University Plan and National Science Council, Taiwan

Grant number: YM-101AC-B3 and NSC 100-2314-B-010-020-MY2

Correspondence:

Cheryl C. H. Yang

Institute of Brain Science, National Yang-Ming University, No. 155, Sec. 2, Li-Nong St., Beitou, Taipei 11221, Taiwan

Tel: +886-2-28267058 Fax: +886-2-28273123

e-mail address: cchyang@ym.edu.tw

Copyright © 2014 by the American Physiological Society.
Involuntary exercising rats undergo more physical and mental stress than voluntary exercising rats; however, these findings still lack electrophysiological evidence. Many studies have reported that theta rhythm appears when there is mental stress and that it is affected by emotional status. Thus, we hypothesized that the differences between voluntary and involuntary movement should also exist in the hippocampal theta rhythm. Using the wheel and treadmill exercise models as voluntary and involuntary exercise models, respectively, this study wirelessly recorded the hippocampal electroencephalogram, electrocardiogram, and three-dimensional accelerations of male young rats. Treadmill and wheel exercise produce different theta patterns in the rats before and during running. Even though the waking baselines for the two exercise types were recorded in different environments, there do not exist any significant difference after distinguishing their sleep/wake status. When the same movement-related parameters are considered, the treadmill running group showed more changes in their theta frequency (4-12 Hz), in their theta power between 9.5-12 Hz, and in their heart rate than the wheel running group. A positive correlation between the changes in high frequency (9.5-12 Hz) theta power and heart rate was identified. Our results reveal various voluntary and involuntary changes in hippocampal theta rhythm as well as divergences in heart rate and high frequency theta activity that may represent the effects of an additional emotional state or the sensory interaction during involuntary running by rats.

**Keywords:** wheel running; treadmill running; hippocampal theta rhythm; physical activity; heart rate
Introduction

Theta band oscillations (4-12 Hz) are prominently in the hippocampus during locomotor activity and can be recorded easily (Kahana et al. 2001; Vinogradova 1995); it has been suggested that these code for information on motor behavior (Shin and Talnov 2001). Studies have suggested that different types of locomotion lead to different hippocampal theta patterns (Arnolds et al. 1984; Gengler et al. 2005; Sinnamon 2005). Furthermore, theta activity also represents various special cognitive functions and/or special learning related functions, therefore, an increase in theta power may be related to higher cognitive functioning and special learning functions (Cummins and Finnigan 2007; Klimesch 1999; Landfield et al. 1972; Laukka et al. 1995; Olvera-Cortes et al. 2004). Many extrinsic and intrinsic factors modulate/influence theta rhythm during behavior testing, but these findings have been inconsistent if not contradictory. Some studies have implied that the recent past experience of the animal is able to affect theta rhythm (Hinman et al. 2011; Villarreal et al. 2007).

In relation to the behavior of animals, two diverse types of hippocampal theta rhythm have been proposed: a cholinergic-dependent activity that ranges from 4-7 Hz (immobility-related theta) and a non-cholinergic component that ranges from 6-9 Hz (voluntary motor theta or cognition-related theta) (Kramis et al. 1975; Olvera-Cortes et al. 2004; Vanderwolf and Baker 1986). Type 1 theta is related to movement, and type 2 theta involves sensory processing. During voluntary motor behaviour, both type 1 and type 2 theta activity are active, and theta frequency is increased. Furthermore, an exploration-related sub-band of theta frequency ranging from 5-12 Hz has also been postulated to exist (Gavrilov et al. 1996; Hinman et al. 2011; Vinogradova 1995). Therefore, it would seem that the complete theta window (4-12 Hz) should be divided...
into a number of different frequency sub-bands according to the above subdivisions of the theta
rhythm (Gutierrez-Guzman et al. 2011; Olvera-Cortes et al. 2002). On the other hand, the
relationship between hippocampal theta and voluntary movement and/or spatial learning has
been established, yet the possibility that visual modulation of theta oscillation is correlated with
these processes has not been clarified. Thus, when studying the hippocampal theta rhythm, it is
important during the recording to exclude visual information effects and control environmental
light.

Treadmill running and wheel running are the most commonly used exercise models when
studying rodents. Treadmill exercise with defined exercise parameters (intensity, duration, etc)
can be considered an involuntary exercise model. Wheel exercise, in which the rats can run
freely in their cages, can be considered a voluntary model. Compared to the treadmill running
(involuntary exercise model), the wheel running (voluntary exercise model) causes lower levels
of stress. Involuntary exercise results in a higher level concentration of serum corticosterone
(Brown et al. 2007; Ke et al. 2011; Ploughman et al. 2007), but also results in reduced spatial
learning and aversive memory after long-term training, when compared to voluntary exercise.
Since treadmill exercise causes a wide range of neuronal responses that may be related to stress,
previous studies have identified that these two exercise model result in different types of brain
functioning (Leasure and Jones 2008; Lin et al. 2012; Ploughman et al. 2005). Only one study
has used both forced and spontaneous running to explore theta rhythm, but this study did not
discuss the different effects of the two running models on theta rhythm; furthermore, they also
failed to consider the waking baseline value of the theta rhythm. Thus their results cannot be
In this study, we compared voluntary and involuntary exercise using the wheel and treadmill models. We have established a free moving rat model that can be used to study various cerebral mechanisms simultaneously during treadmill exercise and wheel running, even in a pipe (Chen et al. 2011; Kuo et al. 2011; Kuo et al. 2010b; Li et al. 2012; Li et al. 2008). The rats can run freely without physical restraint; in this context any limits to their movement could result in a discontinuity of motion. We hypothesize that voluntary and involuntary exercise cause different patterns of theta rhythm before and during running. The different sensory stimuli from different types of exercise may affect theta rhythm. The aims of the present study are as follows: firstly, to determine whether voluntary and involuntary exercise cause different changes in theta rhythm, both before and during running; secondly, to compare the two running models when the rats have the same movement-related parameters (physical activity; speed; heart rate); and thirdly, to explore the relationship during running between theta rhythm and heart rate and between theta rhythm and physical activity.

Material and Methods

The experiments were carried out on 8 weeks old male Wistar-Kyoto rats (WKY, n=24). The rats were raised in a sound-attenuated room under a 12:12-h light-dark cycle (11:00-23:00 lights on) at a controlled temperature (22 ± 2°C) and humidity (40-70%). The detailed surgical procedures have been described previously (Kuo et al. 2004b; Li et al. 2008). On the day of electrode implantation, the rats were 7 weeks old. Under pentobarbital anesthesia (50 mg/kg, i.p.), each rat
was placed in a standard stereotaxic apparatus, and the dorsal surface of the skull was exposed and cleaned. The exposed skull surface had seven screws fixed into it. The electroencephalogram (EEG) was recorded by screws fixed into skull, one of which served as the occipital electrode (2 mm lateral and 2 mm anterior to lambda), while another posterior to lambda acted as the reference electrode. A monopolar electrode made with 230 μm diameter Teflon-insulated stainless steel wire was stereotaxically and permanently implanted into the right hippocampal CA1 region (2.4 mm lateral and 4.3 mm posterior to bregma, and 2.4 mm from dura). The electromyogram (EMG) was recorded from two electrodes inserted into the dorsal neck muscles, and the electrocardiogram (ECG) was recorded from two electrodes placed dorsally under skin. All the electrophysiological signals were joined to a connector fixed to the skull by dental acrylic. After surgery, the rats were treated with an antibiotic (chlortetracycline) and individually housed in translucent cages for 1 week to recover.

After they had recovered, the rats in the voluntary running group were recorded to obtain 24-h baseline values using identical cages. Before the each recording, the rats were held to allow the wireless sensor to be fixed onto the connector of their head. Then we added a running wheel (2154F0105, wheel Ø: 34.5 cm; Techniplast, Buguggiate, Italy) for voluntary exercise (n=9) to identical cages during the early light period. The voluntary group did not have an acclimation period, but they were housed with a wheel for at least 12 hours to allow them to become familiar with it. The recording of the voluntary running was started at the beginning of the dark period and covered over 24-h of recording (at least 3 consecutive days). In order to exclude possible training effects and/or visual information on the theta rhythm, we only analyzed the 1st (4 rats) and 2nd (5 rats) wheel runs of the day and the dataset analyzed was for the last dark period.
Running head: The EEG changes during wheel and treadmill running

(8:00-11:00 AM) only. Since running behavior is very complicated and individual differences must exist, we set the threshold for running speed at 5 revolutions per minute (rpm) and the minimal running duration at that speed or higher was set at 20 s (Li et al. 2012).

In contrast to the above, the involuntary exercise used a treadmill model (n=15). After the surgery and recovery, the rats of involuntary running group were housed individually and had their 24-h baseline values recorded in their own cages. Before the involuntary running test, the rats were familiarized with the motorized treadmill (Model T510E, Diagnostic & Research Instruments CO., Taoyuan, Taiwan) by running on the treadmill for 10 min on 3 consecutive days (belt speed = 8 m/min). On the recording day, the rats were held to fix the wireless sensor onto their head and placed in the treadmill during the last dark period (09:00-11:00 AM). All electrophysiological signals were recorded for 80 min. 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 this was followed by 30-min recovery. To exclude the possible effects of fatigue and different running statuses (Kuo et al., 2011), we only chose the first 5 min of treadmill running and exclude the first 24 s of the initiation stage of running. After the running, we also recorded 24-h of signals in their own cages and started the recording before light-on. The treadmill belt was able to reach its default speed value within 1 s. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Yang-Ming University. The IACUC permit number was 981238. Rats were handled following the European Communities Council Directive of 24 November 1986 (86/609/EEC) for the use of animals in research.
Data acquisition and storage were similar to those of the procedure described previously (Chen et al. 2011; Kuo et al. 2004a; Kuo et al. 2010b; Kuo et al. 2004b; Li et al. 2008). A wireless sensor (25×21×18.5 mm, 8.3 g, KY4C, K&Y Lab, Taiwan) was mounted on the head of the rat and this was used to acquire ECG, EEG, EMG, and 3-dimensional acceleration. All electrophysiological signals were amplified 1000-fold. Three-axis accelerations were detected by a triaxial accelerometer (ADXL330, Analog Devices Inc., Norwood, MA, USA) in the anteroposterior (X), mediolateral (Y), and vertical (Z) dimensions, each detecting acceleration from -3 to +3 gravity (G). The EEG and accelerations were differentially filtered (EEG: 0.16 to 48 Hz, Acceleration: DC to 29 Hz) and were synchronously digitized by an analog-digital converter with sampling rate of 125 Hz and 62.5 Hz, respectively. All digitized signals were then wirelessly transmitted to a nearby data recorder (KY3, K&Y Lab, Taiwan) in which they were stored on a flash memory for off-line analysis.

We designed a special computer program in Pascal language (Borland Pascal 7.0, Borland, USA) for bioelectric signals (Kuo et al. 2012; Kuo et al. 2004a; Kuo et al. 2010a; Kuo et al. 2004b; Kuo et al. 2008; Kuo and Yang 2005). The digital signal processing of the bioelectric signals was similar to the procedures used in our previous studies (Kuo et al. 2004a; Kuo et al. 2004b). We use a 2-s window length in order to provide a frequency resolution of 0.5 Hz, which is necessary to study the three sub-bands of theta power. The EEG was resampled at 64 Hz and was truncated into successive 2-s time segments (windows or epochs). 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 the attenuation resulting from the
sampling and the application of the Hamming window. For each time segment, we calculated the amplitude power of EEG within the theta range (total power, TP, 4-12 Hz.), and mean power frequency of EEG within the theta range (Frq). Based on evidence from the sensorimotor integration model (Bland and Oddie 2001; Bland and Vanderwolf 1972; Cruikshank et al. 2012), the theta frequency range was divided into three sub-bands: low frequency (LT, 4-6.5 Hz), middle frequency (MT, 6.5-9.5 Hz), and high frequency (HT, 9.5-12 Hz) (Gutierrez-Guzman et al. 2011; Olvera-Cortes et al. 2002).

We used the EEG and EMG signals to score sleep status. Sleep analysis was carried out according to our previously developed and semi-automatic computer procedure, which has been described in detail elsewhere (Kuo et al. 2004a; Kuo et al. 2004b; Kuo et al. 2008; Kuo and Yang 2005). The procedure discriminates the consciousness states into active waking, quiet sleep, and paradoxical sleep. Briefly, continuous power spectral analysis was applied to the EEG and EMG signals, from which the mean power frequency (MPF) of the EEG and the power magnitude of the EMG were quantified. The sleep-wake stage was defined as active waking if the corresponding MPF was greater than a pre-defined MPF threshold ($T_{MPF}$) and the EMG power was greater than a pre-defined EMG power threshold ($T_{EMG}$); as quite sleep if the corresponding MPF was less than the $T_{MPF}$ and the EMG power was less than the $T_{EMG}$; and as paradoxical sleep if the corresponding MPF was greater than the $T_{MPF}$ and the EMG power was less than the $T_{EMG}$. We only selected 3-5 min of active waking status to represent the baseline values while the rats were usually moving or walking around. To exclude the possible effects of circadian rhythm, we only chose the waking baseline values in the individual cages during the latest 3-h of dark period. When considering the waking baseline values in the exercise
Running head: The EEG changes during wheel and treadmill running

environment, we also collected 3-5 min of the waking status from the latest 3-h of dark period in
the voluntary group and selected the waking status before running in the involuntary group.

The analysis of physical activity was modified from previous studies (Bouten et al. 1994; Plasqui
and Westerterp 2006). A vector magnitude of the three dimensional accelerations was calculated
using $\sqrt{X^2+Y^2+Z^2}$. Valid vibrations were counted separately for the three dimensions and the
vector magnitude is presented as the unit of count per minute (cpm). The threshold for each
count was set at 0.08 gravity. The vibration of the vector magnitude was regarded as the physical
activity.

We used a light sensor to measure the wheel speed. There are four apertures on the wheel. When
the light enters to the aperture, the sensor will not be reflected. The sensor detects and counts the
number of losses of reflection from the wheel. Each count of four represents a revolution of
wheel. We used the reciprocal of the count interval to represent rpm.

Once the experiments had been completed, the rats were placed under deep anesthesia with
overdose of pentobarbital and perfused transcardially with 0.9% saline, followed by 4%
formalin. The brains were preserved in 30 % sucrose solution, then sectioned and stained with
cresyl violet to confirm the hippocampal electrode position.

The distributions of the theta power of the hippocampal EEG spectrogram were not normal but
were skewed to the right; therefore they were logarithmically transformed to correct the
skewness of the distribution (Kuo et al. 2010b; Polunina and Davydov 2004). The effects of
Running head: The EEG changes during wheel and treadmill running

exercise and time 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 Fisher’s least significant difference test. Comparisons between two sets of data were carried out by the Student’s t test. Comparisons of the temporal alterations in each parameter were analyzed using repeated measures ANOVA. Statistical significance was assumed for \( p < 0.05 \). Values are expressed as mean ± SEM. Differences between values and zero were assessed using a 95% confidence interval analysis.

Results

This study attempted to compare the differences in hippocampal theta rhythm, which including frequency and various different ranges of theta power, between involuntary and voluntary running using the chronological order of the running or the same movement-related parameters during running (physical activity; speed; heart rate). Pictures of wireless sensor, connector, and the anatomical location of the recording electrode are shown in figure 1. The raw signal tracings of EEG, ECG, and three dimensional accelerations of a rat before and during (8-16 sec) running are shown (Fig. 2). In part I, we explore the changes in hippocampal theta rhythm with respect to the chronological order of treadmill and wheel running over 32 s than includes the start of running at 18 s. In order to explore the continuous changes in each parameter before and during running and to achieve a higher temporal resolution, the analysis used a 2-s epoch, which allows better quantitative analysis (Fig. 3). To exclude the possible effects of learning or novel environment on theta rhythm, we compared theta rhythms between 1st and 2nd wheel running day, and no significant differences on theta rhythm was found (Fig. 4) between these two days. Without considering physical activity and speed, treadmill and wheel running showed different
patterns for the theta rhythm before and during running. Before running (Fig. 3), wheel running group carried out more physical activity, had a faster heart rate, and had higher Frq, TP and MT than the rats in the treadmill running group. Even at the first running point, the MT in the wheel running group was still higher than that in the treadmill running group. The changes in Frq, LT and MT were only obvious before wheel running. Both the treadmill and wheel running groups had lower LT values during running, but the running-related change in LT for the treadmill running group occurred later. During running, the absolute values of Frq in wheel running group were found to be significantly lower than those of the treadmill running group. In order to diminish any effects caused by individual differences, all parameters minus the mean values for the first 6 s of running were calculated to give change values for the three parameters, namely ΔFrq, ΔTP and ΔMT. These values in the wheel running group are lower than those in the treadmill running group (Fig. 3B). The last point of the ΔLT before running in the wheel running group is significantly lower than that in the treadmill. In contrast, the ΔLT at second 26, second 28 and second 32 are significantly higher than those at the same time points in the treadmill running group. The ΔHT at the first point of the treadmill running is dramatically higher than the value at the first point of the wheel running. It is important to note that this is only true for the first run on either the wheel or the treadmill. The changes in physical activity and heart rate in the treadmill running group are also significantly higher than those in the wheel running group (Fig. 3B). However, considering the chronological order with this window length, it is not possible to distinguish the sleep/wake status and may have been an intermix in sleep and wake status before running, especially in involuntary group.
In part II, we explore the differences in hippocampal theta rhythm, physical activity and heart rate between the treadmill and wheel running groups when the physiological parameters (physical activity; speed; heart rate) are the same for these two running groups. To exclude the possibility of fatigue among the treadmill running group, we only analyzed the first 5 minutes of treadmill running and excluded the initiation stage, based on the results of our previous study (Kuo et al. 2011). We selected the lasted 3-h of dark period as the waking baseline to exclude any possible effects of the rats' circadian rhythm; furthermore, the analysis of the baseline values took place in the identical cages and in the same exercise environments. After scoring the sleep/wake status using the EEG and EMG signals, we found there was no significant difference in their waking baseline values between in the cages and in the exercise environments. It was also found that there was no significant difference in their waking baseline values between in the treadmill and in the cage with wheel (Fig. 5A). When the physical activity levels are the same, both treadmill and wheel running show elevated Frq, TP, MT and HT of the theta rhythm, decreased LT of the theta rhythm and increased physical activity compared with awake baseline (Fig. 5). When the running speeds are the same, Frq, TP and MT of the theta rhythm and physical activity are elevated during running compared with awake baseline. Moreover, the increments in Frq and physical activity during wheel running are less than during treadmill running when the rats perform at the same speed running (Fig. 5). When the two groups have the same heart rate during running, the Frq, TP and MT of the theta rhythm and physical activity during wheel running are significantly more than during the awake baseline. However, wheel running shows a decreased LT of the theta rhythm when the rat was performing with the same HR as a treadmill running rat. The Frq during wheel running was also found to undergo less change than during treadmill running (Fig. 5). Finally, it's interesting to note that the wheel
running rats do not have a significantly elevated heart rate (Fig. 5B), even when the physical activity, speed and heart rate during running in wheel running group are similar to those in treadmill running group.

Both treadmill and wheel running result in significant changes in Frq, TP, LT and MT of the theta rhythm and physical activity. However, when the wheel running rats were performing at the same physical activity as the treadmill rat, then the HT of the theta rhythm also shows a significant increase. When the wheel running rats were performing at the same speed or heart rate as the treadmill running rats, the ΔFrq and ΔHT are less than treadmill running groups (Fig. 5B). This phenomenon also existed when the relationship between ΔHR and ΔHT is explored. Figure 6 shows that there is a positive correlation between ΔHR and ΔHT only when these two exercise models have the same speed or heart rate during running (r=0.468, p=0.033; r=0.443, p=0.039). However, changes in any other of theta rhythm parameters were not correlated with ΔHR or with ΔPA.

**Discussion**

Our study has established a platform for comparing the effects of voluntary and involuntary running on theta rhythm, heart rate and physical activity. We used the wheel and treadmill running models as systems that allow voluntary and involuntary movement, respectively. We found that wheel and treadmill movement showed different changes in theta rhythm, physical activity and heart rate before running, which may be a result of not distinguishing the sleep/wake status. We considered that sleep/wake status is the main factor causing the obvious differences before running in these two groups. When the chronological order of running was examined,
both wheel and treadmill running group have the increases in theta rhythm, heart rate and physical activity. It is interesting to note that the high frequency of theta during treadmill running is obviously higher than during wheel running, but only at the first time point of running; however, at this same time point, the change in physical activity was not significant between the two exercise models. On the other hand, when we examine the results for the same speed or heart rate during running without considering the chronological order of running, the Frq during wheel running is less than during treadmill running. At the same speed, wheel running rats show less physical activity. After deducting the waking baseline, the treadmill running group was found to undergo more changes in Frq and HT of the theta rhythm and heart rate compared to the wheel running group when they had the same running speed and when they had the same heart rate.

This study is the first study to compare the differences in hippocampal theta rhythm between voluntary and involuntary movement, and, furthermore, we also recorded physical activity and heart rate, which offers additional information that helps to discriminate voluntary running from involuntary running. This allows the potential relationships that occur in voluntary/involuntary movement and EEG to be explored.

Unlike humans, rats sleep both during the day and night with frequently sleep/wake changes when free moving (Borbely 1976; Kuo et al. 2004a; Kuo et al. 2004b; 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 status, especially during the baseline period, the sleep or waking transitions themselves may produce larger fluctuations than the test stimulation such as exercise. EEG activities also vary with the circadian rhythm (Grasing
and Szeto 1992; Yasenkov and Deboer 2010), thus we only selected and compared the waking baseline values captured during the latest 3 hours of the dark period.

It's well known that both mental and physiological stresses are able to change heart rate (Hjortskov et al. 2004; Sandercock et al. 2005; Sandvik et al. 1995). Compared with an animal undertaking voluntary movement, involuntary running animals suffer more stress, especially psychological stress (Leasure and Jones 2008; Ploughman et al. 2007). During forced exercise the rat is unable to be in control of the speed and duration of movement. In present study, members of the involuntary group are placed in the limited space before running, but voluntary group remained free in their own cage, which has a relatively large amount of free space to move in. In addition, the rats of two groups were also residing in different environments. Although we did not find any significant differences in the rats' awake baseline values between the different exercise environments, we cannot exclude that there may exist other possibilities of baseline values for the different durations of familiar treatment. In terms of chronological order, we cannot control the sleep/wake status before running. Since the rats of involuntary group were placed in treadmill over 30 min before running, the rats may have fallen asleep for 5-10 min during this period. On the contrary, the rats in the voluntary group were able to run freely around the cage and were almost always active before running. Thus, our results may have been affected by the fact that the voluntary group has higher physical activity and a faster heart rate than involuntary group before running. We considered that the sleep/wake status of the rats before exercise is the main factor causing the obvious differences before running in the two groups. When considering the same movement-related parameters, running is able to elevate physical activity in these two exercise models. In order to exclude the effects of sleep/wake status on all
parameters, we considered possible movement-related parameters and compared the two exercise types (Fig 5). Although we are unable to exclude the possible effects of environmental enrichment, the similarity of the baseline values in their own cages and in the exercise environments support the idea that the differences between two groups are not caused by the rats' divergent environments and treatments to any large extent. Therefore, the influences of environment or treatment over theta rhythm are likely to be relatively small in this study. Nevertheless, the wheel running group shows fewer changes in heart rate across all situations than the treadmill running group. These results suggest that there exist other factors that are causing the increment in heart rate other than physiological stress.

Low frequency theta activity is immobility-related theta and can be recorded during a "cognitive" task (Olvera-Cortes et al. 2004; Vanderwolf 1969; Vanderwolf and Baker 1986). In this study, we found LT diminished before wheel running and was maintained at its lower value during running. However, physical activity did not show a significant change prior to voluntary movement. This prior drop of LT may indicate that the rat is preparing to move. We also determined that both voluntary and involuntary movement are able to cause significant LT reductions, but the drop of LT during voluntary running was not greater than that during involuntary running. Although there did not exist any significant differences in LT for the waking baselines of the two exercise type, we cannot exclude the effect of the distinct environments. Compared with treadmill running rats, the wheel running rats ran in the same place, but the view was more extensive. Thus the sensory information is more complicated during wheel running than during treadmill running. In order to eliminate the different environments, involuntary movement needs to be performed on a wheel with automatic rotation.
function. However, the limited space present in an automatic wheel is another confounding factor. On the other hand, 6-9 Hz theta is known as a non-cholinergic component and can be also defined as voluntary motor theta or cognition-related theta (Kramis et al. 1975; Olvera-Cortes et al. 2004; Vanderwolf and Baker 1986). Our previous study has revealed that MT is positively related to speed and physical activity (Li et al. 2012). If we compare the status before running in these two exercise models (Fig. 3) without scoring the sleep/wake status, the voluntary group has more MT and physical activity simultaneously than the involuntary group. After discriminating the sleep/wake status, the waking baseline values for MT and physical activity in the two groups are similar. Thus, MT ranging from 6.5-9.5 Hz resembles movement-related theta wave.

Based on our previous studies, we have proposed that the 6-10 Hz range of theta frequency is correlated with exercise effort (Kuo et al. 2011; Li et al. 2012). In a human study, theta frequency has been shown to also be linked to mental effort (Mizuki et al. 1992; Onton et al. 2005). In this study, the theta frequency was divided into three ranges. We found that high frequency theta power in the treadmill running group showed the highest values at the first running point and that these changes were positively correlated with the changes in heart rate. Previous studies have determined that the elevated theta frequency represents the sensory input for the initiation and maintenance of movement is commonly seen during the initiation of movements (Bland et al. 2007; Bland et al. 2006). Our previous study used 4-s resolution and found that both theta frequency (6-10 Hz) and physical activity showed elevated values during the initiation stage of running. In the present study, we explore the complete theta window (4-12 Hz) and found that these additional increases in heart rate among the treadmill running group rats may be induced by psychological factors and not by physical movement or activity. Since the
rats must assess the timing and speed of running when treadmill starts, these differences of heart
rate and theta rhythm could reflect complex sensory input in the involuntary condition. Type 2
theta can be recorded during the shock probe test since shock was related to the initiation of an
avoidance response (Bland et al. 2007). Thus the additional sensory drive in the involuntary
condition may represent as one of stressful input. We considered that high frequency theta
activity is likely to be associated with involuntary movement-related mental stress.

By EEG recording, this study was able to explore the difference between voluntary and
involuntary movement and we examined the role of theta rhythm in locomotion. However, we
are unable to offer in this study any biochemical parameters that are known to be associated with
mental stress; such markers would help to directly reveal the fact that high frequency theta
activity is related to mental stress. Previous studies have discussed the long-term effects on stress
responses of voluntary and involuntary movement by sacrificing the animals and analyzing their
serum corticosterone concentrations (Brown et al. 2007; Ke et al. 2011; Ploughman et al. 2007).
Since different running loadings existed in these two exercise types, comparing the
corticosterone concentrations between the two groups is not easy in this study. Changes in theta
rhythm are considered be related to emotional state by various authors (Mitchell et al. 2008;
Yamamoto 1998). When suffering different types and levels of stress, the high frequency and
low frequency components of theta will be changed. Many studies have reported that there is a
relationship between hippocampal theta wave, various types of animal behavior and the septo-
hippocampal pathway (Dutar et al. 1995; Gilad et al. 1985; Vanderwolf 1969). Therefore, the
differences in the effect of these two exercise models on hippocampal theta rhythm may mean
that there exists an emotional discrepancy among between the two types of movement. Based on
the above, the underlying mechanisms associated with the relationship between theta rhythm and voluntary/involuntary movement warrants further exploration.

Conclusion

Voluntary and involuntary running show distinct theta patterns before and during running. Involuntary running cause increases in theta frequency, high frequency theta activity, and heart rate compared to voluntary running. These discrepancies in high frequency theta activity and heart rate between two exercise groups may represent the emotional status or the sensory interaction that occurs during running by the rat.

This study was supported by a grant (YM-101AC-B3) from the Ministry of Education, Aim for the Top University Plan and grant (NSC 100-2314-B-010-020-MY2) from National Science Council, Taiwan.


Kuo TBJ, Li JY, Hsieh SS, Chen JJ, Tsai CY, and Yang CCH. Effect of aging on treadmill exercise induced theta power in the rat. *Age (Dordr)* 32: 297-308, 2010b.


Vanderwolf CH, and Baker GB. Evidence that serotonin mediates non-cholinergic neocortical low voltage fast activity, non-cholinergic hippocampal rhythmical slow activity and contributes to intelligent behavior. *Brain Res* 374: 342-356, 1986.


Yamamoto J. Relationship between hippocampal theta-wave frequency and emotional behaviors in rabbits produced with stresses or psychotropic drugs. *Jpn J Pharmacol* 76: 125-127, 1998.

**Figure Captions**

**Fig. 1** A. Picture of the wireless sensor and the connector on the head of the rat. B. Picture of handmade connector with electroencephalogram (EEG), electromyogram (EMG) and electrocardiogram (ECG) electrodes. Anatomical location of the recording electrode (C, D). C. Photomicrograph of a coronal section taken through the hippocampus. Arrow indicates the location of the electrode tip. D. Diagrammatic representation of the hippocampus, showing electrode locations of the wheel (closed circles) and treadmill (open circles) groups. DG: dentate gyrus.

**Fig. 2.** Raw signal tracings of electroencephalogram (EEG), electrocardiogram (ECG), and three dimensional accelerations of a rat before and during (8-16 sec) running (Wheel/Treadmill).

**Fig. 3.** Temporal alterations (A) and changes, Δ, (B) in frequency (Frq, 4-12 Hz), total power (TP, 4-12 Hz), low frequency power (LT, 4-6.5 Hz), middle frequency power (MT, 6.5-9.5 Hz) and high frequency power (HT, 9.5-12 Hz) of the hippocampal theta rhythm, physical activity (PA) and heart rate (HR) using a 2-s resolution during 32 s that included treadmill running and wheel running (18-32 s) by rats. The values had the mean values for 0-6 s subtracted to give the change values (Δ). The vertical lines indicate the start of running. Data are expressed as mean ± SEM of 9 or 15 rats. *p < 0.05 vs. treadmill, † p < 0.05 vs. the mean values for 0-6 s in treadmill group, # p < 0.05 vs. the mean values for 0-6 s in wheel group. ln indicated natural logarithm.
Fig. 4. Changes in frequency (Frq, 4-12 Hz), total power (TP, 4-12 Hz), low frequency power (LT, 4-6.5 Hz), middle frequency power (MT, 6.5-9.5 Hz) and high frequency power (HT, 9.5-12 Hz) of hippocampal theta rhythm in 1st and 2nd wheel running day. Values are means ± SEM of 4 or 5 rats. ln indicated natural logarithm.

Fig. 5. The values (A) and changes (B) in frequency (Frq, 4-12 Hz), total power (TP, 4-12 Hz), low frequency power (LT, 4-6.5 Hz), middle frequency power (MT, 6.5-9.5 Hz) and high frequency power (HT, 9.5-12 Hz) of hippocampal theta rhythm, physical activity (PA) and heart rate (HR) during the waking baseline in the cage (Bas in cage), during the waking baseline in treadmill/wheel (Bas) and during the different running states (Treadmill; with the same PA in wheel, the same PA; with the same speed in wheel, the same speed; with the same HR in wheel, the same HR). Values are means ± SEM of 9 or 15 rats. #p < 0.05 from vs. Bas in cage, *p < 0.05 from vs. Bas, †p < 0.05 vs. treadmill. ln indicated natural logarithm.

Fig. 6. The correlation coefficients between the changes (Δ) from the waking baseline for the high frequency of hippocampal theta rhythm parameters (HT) and Δ heart rate (HR) relative to the Δ physical activity (PA) of the rats during voluntary (wheel) and involuntary (treadmill) running over the dark period. Values are means ± SEM of 24 rats. ln indicated natural logarithm.
Fig 2

Running

Wheel

Treadmill
The same PA

\[ \Delta HT \approx \ln(\mu V^2) \]

\[ r = 0.109 \quad \rho = 0.612 \]

\[ r = 0.103 \quad \rho = 0.631 \]

The same speed

\[ \Delta HT \approx \ln(\mu V^2) \]

\[ r = 0.468 \quad \rho = 0.033^* \]

\[ r = 0.180 \quad \rho = 0.436 \]

The same HR

\[ \Delta HT \approx \ln(\mu V^2) \]

\[ r = 0.443 \quad \rho = 0.039^* \]

\[ r = -0.115 \quad \rho = 0.600 \]