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Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats

Abstract: Sleep disorders cause cognitive dysfunction in which impaired neuronal plasticity in the hippocampus may underline the molecular mechanisms of this deficiency. As sirtuin 1 (SIRT1) plays an important role in maintaining metabolic homeostasis and neuronal plasticity, this study is aimed to determine whether melatonin exerts beneficial effects on preserving SIRT1 activation following total sleep deprivation (TSD). TSD was performed by disc on water method for five consecutive days. During this period, animals daily received melatonin at doses of 5, 25, 50 or 100 mg/kg. The cytochrome oxidase (COX) histochemistry, SIRT1 immunohistochemistry together with Morris water maze learning test were performed to examine the metabolic, neurochemical, as well as the behavioral changes in neuronal plasticity, respectively. The results indicate that in normal rats, numerous COX and SIRT1 positive-labeled neurons with strong staining intensities were found in hippocampal pyramidal and granular cell layers. Following TSD, both COX and SIRT1 reactivities were drastically decreased as revealed by reduced staining pattern and labeling frequency. Behavioral data corresponded well with morphological findings in which spatial memory test in water maze was significantly impaired after TSD. However, in rats receiving different doses of melatonin, both COX and SIRT1 expressions were successfully preserved. Considerably better performance on behavioral testing further strengthened the beneficial effects of melatonin. These findings suggest that melatonin may serve as a novel therapeutic strategy directed for preventing the memory deficits resulting from TSD, possibly by effectively preserving the metabolic function and neuronal plasticity engaged in maintaining cognitive activity.

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Introduction

It is well established that a good night's sleep exerts restorative functions on our body and mind, resulting in a sense of well-being and facilitation of neuronal plastic changes that underlie the molecular mechanisms of learning and consolidation of memory [1–3]. Chronic sleep loss or sleep deprivation (SD) cause desynchronization of cellular function, which unavoidably leads to a variety of neurobiological, physiological, metabolic and cognitive deficiencies [4–8]. Previous studies have indicated that cognitive deficits may result from sleepless-induced remodeling of brain regions that participate in the formation of anxiety, executive function and memory [5, 9, 10]. Recent reports also demonstrated that altered synaptic, metabolic, or membrane excitability may serve as underlying molecular bases responsible for the development of neuro-plastic and neuro-behavioral changes [11–13]. It is suggested that well-regulated neuronal plasticity is essential for performing normal cognitive activities [14]. Effectively preserving the mechanisms involved in maintaining the plastic homeostasis may thus be of great importance for individuals to create a healthy and productive lifestyle.

The 'longevity protein' sirtuin 1 (SIRT1) is a member of NAD^+-dependent histone/protein deacetylases capable of promoting the cellular lifespan through a series of mechanisms involving in genome stability and plastic homeostasis [15, 16]. During the past few years, a variety of studies had demonstrated that induction of SIRT1, either by sirtuin activators such as resveratrol, or metabolic conditioning associated with calorie restriction (CR), would silence chromatin, reduce spontaneous DNA mutations and enable the brain to preserve synaptic strength and neuronal integrity [17–20]. It has been reported that SIRT1 displays its protective role in several models of neurocognitive diseases [21–25]. These studies indicated that SIRT1 regulates the neuronal plasticity under challenging conditions that may lead to oxidative stress and cognitive impairments [21]. Recent reports also documented that activation of SIRT1 defends against the Alzheimer's disease (AD), Huntington's disease, amyotrophic lateral sclerosis as well as the prion-mediated neuro-pathogenesis [22–25]. It is shown that the mechanism of SIRT1 activation is largely depended on its regulatory abilities to modulate transcriptional factors such as NF-κB, p53, FOXO, PPARγ (and its co-activator PGC-1α), and even uncoupling proteins

(UCPs) that control normal gene expressions and apoptosis important for metabolic homeostasis and neuronal plasticity [17, 18, 20, 26]. Activation of SIRT1 also promotes the nonamyloidogenic processing of amyloid precursor protein (APP) through inhibition of FOXO3a-mediated ROCK1 gene expression, therefore reducing the AD amyloid-β peptide (Aβ) generation and preserves the spatial reference memory [27]. Given that impaired neuronal plasticity of the hippocampus may underlie the potential mechanisms leading to cognitive deficiency following SD [9–13], effectively preserve SIRT1, either by internal activation or treatment molecules that increase its activity, may thus represent a novel and significant approach to counteract the SD-related neuropathological sequelae.

Melatonin, the chief secretory product of pineal gland, is best known for its effects on circadian rhythmicity, immune function, free radical scavenging and anti-oxidative activity [for review, see 28, 29]. Within the past few years, the functional role of melatonin in improving cognitive activity has gradually been reported [30, 31]. These studies indicated that melatonin may serve as an endogenous synchronizer that stabilizes the temporal order of bodily and mental experiences [32]. Electrophysiological studies also suggest that melatonin is a potential regulator involved in the processes of memory formation, long-term potentiation (LTP), synaptic transmission, as well as neuronal plasticity in hippocampus and other brain regions [33–35]. It is implied that melatonin exerts its neuroprotective effects on cognitive function through cytoskeletal modulation and second-messenger signal transduction pathway-related gene expression mechanisms [36, 37]. By effectively protecting the cytoskeletal organization and spatio-temporal integration of mechanisms involved in homeostatic regulation of synaptic plasticity, application of melatonin may therefore be a valuable means to enhance the well-maintenance of learning and higher conscious activity [36–38].

However, although the positive effects of SIRT1 in modulating the cellular metabolic homeostasis and neuronal plasticity are well-documented, the potential alterations of SIRT1 in the hippocampus following SD, a harmful condition known to damage the specific region that participates in cognitive activity, has not yet been reported. Moreover, whether exogenous melatonin administration after SD would significantly improve the neuronal plasticity that underlies the molecular mechanism of cognitive regulation still remains to be explored. To answer these questions, this study is designed to examine the possible neuronal plastic changes induced by SD through metabolic, neurochemical, as well as the behavioral approaches by the use of cytochrome oxidase (COX, an endogenous metabolic marker closely reflects the neuronal activities involved in learning and memory [39, 40]) histochemistry, SIRT1 immunohistochemistry, and Morris water maze test, respectively. In addition, as SD has long been regarded to exert some extent of stress on experimental animals [41], two distinguished stress biomarkers plasma corticosterone and ACTH levels were further detected to elucidate the possible effects of stress, other than SD, on cognitive activities [42]. Considering the increased prevalence of traumatic accidents induced by SD-relevant cognitive impairment in this current society [5, 6], exploring the related mechanisms and attempts to provide a potential therapeutic agent will be of great help in diminishing the economic and social costs arising from SD related disability.

Materials and methods

Treatment of experimental animals and surgical procedure

Adult male Wistar rats (n = 72, weighing 200–250 g) obtained from the Laboratory Animal Center of the National Taiwan University were used in this study. All surgical procedures for electroencephalogram (EEG) and electromyogram (EMG) recordings were performed using the well-established methods as described previously [43]. After the operation, all experimental animals were firstly divided into three groups equally (n = 24 for each group). Rats in the first group were subjected to total sleep deprivation for 5 days (TSD group), whereas their yoked control in the second group were housed in the same TSD apparatus but were permitted to sleep (control for sleep deprivation, TSC group). Animals in the third group were kept in a plastic cage placed aside from the TSD apparatus and served as normal untreated controls (Untreated group). During the sleep-deprived period, each of the experimental group (n = 24 per group) was further subdivided into six subgroups with four in each. In the first subgroup, rats did not receive any extra administration of melatonin. In the subgroups II to VI, rats were daily received intraperitoneal injection of Ringer's solution or melatonin at the doses of 5, 25, 50 or 100 mg/kg (body weight), respectively, between 10:00 and 10:30 hr. Melatonin (Sigma, St Louis, MO, USA) was first dissolved in absolute alcohol and then diluted in Ringer's solution with final ethanol concentration less than 1%. During the experimental period, all rats were exposed to an automatically regulated light–dark cycle of 12:12 (light on 7:00–19:00 hr) at a constant temperature of 25 ± 1°C. The animals were allowed to food and water ad libitum. In the care and handling of all experimental animals, the Guide for the Care and Use of Laboratory Animals (1985) as stated in the United States NIH guidelines (NIH publication No. 86-23) were followed.

Sleep deprivation process and recordings

Total sleep deprivation was performed by the disc-on-water (DOW) method as described in our previous studies [7, 8]. This method was chosen because it has previously been validated as able to produce effective TSD in one animal without excessive physical exertion, where as its yoked control (TSC group) has an acceptable amount of sleep in spite of receiving the same activity [44]. Briefly, the apparatus was comprised of two rectangular plastic chambers placed side by side. A single plastic disc serving as rat-carrying platform was built into the lower quarter of the two chambers. Beneath the disc was a rectangular tray filled with water to a depth of 5 cm. Sleep deprivation depended on rats' aversion to water, as rats rarely enter water spontaneously. When sleep onset was detected in the sleep-deprived rat, the disc was rotated slowly at a moderate speed of 3.5 rpm by the computerized

monitoring system, 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 was spontaneously awake, the disc was stationary and the yoked control rat was able to sleep. Electroencephalographic and electromyographic data were recorded and relayed to a computer for digital recording. All the sleep deprivation procedures were further approved by the Laboratory Animal Center Authorities of the Chung Shan Medical University.

Morris water maze learning test

At noontime after 5 days of TSD, all rats were undergone the Morris water maze test for examining the performance of spatial learning and memory [45]. The Morris water maze was consisted of a circular tank (80 cm deep, 164 cm diameter, San Diego Instruments) filled with water to a level of 24 cm. The pool water was made opaque by addition of semi-skimmed milk. The maze was divided into four equal quadrants on the monitoring screen of a computer. An escape platform placed in one of the four quadrants (target quadrant) was submerged 1.5 cm below the water surface. The rats were trained to find the hidden platform according to the spatial cues in the experimental room. Place learning was assessed for eight training trials on the final day of TSD. The maze performance was recorded by a video camera suspended above the maze and interfaced with a video tracking system (San Diego Instruments).

A probe trial was given 12 hr after the training to test the extent of memory consolidation and spatial memory in the water maze. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. In the probe trial, the rat was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The time of crossing the target quadrant and the total time of crossing all quadrants were recorded for 1 min and were used as a measure for spatial learning persistence.

To test the possible deficits in sensorimotor processes after TSD, the rats were tested in the water maze with a visible platform on a new location [46]. Latency times to reach the platform were also recorded for each trial.

Perfusion and tissue preparation

For quantitative histochemical and immunohistochemical studies, rats of all experimental groups were deeply anesthetized with 7% chloral hydrate (0.4 mL/kg) and perfused transcardially with 0.9% saline followed by 300 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. After perfusion, the forebrain segment containing the hippocampus was removed and then immersed in sucrose buffer for cryoprotection at 4°C. Serial 30-μm-thick sections of the hippocampus were cut transversely with a cryostat (CM3050S; Leica Microsystems, Wetzlar, Germany) on the next day and were alternatively placed into two wells of a cell culture plate. Sections collected in the first well were processed for cytochrome oxidase (COX) histochemistry, and those in the second well were processed for sirtuin 1 (SIRT1) immunohistochemistry.

Plasma corticosterone and ACTH assays

The blood samples used for plasma corticosterone and ACTH assays were collected from the left ventricle during the transcardiac perfusion. The blood was 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 was amassed and both plasma corticosterone and ACTH levels were determined by the radioimmunoassay method (corticosterone kit, MP Biomedicals, Costa Mesa, CA, USA; ACTH kit, Nichols Institute Diagnostics, Bad Vilbel, Germany).

Cytochrome oxidase histochemistry

The slightly modified method of Wong-Riley [39] was used to demonstrate the COX reactivity in this study. The reaction medium contained 0.03% cytochrome C (Sigma), 0.05% 3,3′-diaminobenzidine and 0.02% catalase in 0.1 M PB, pH 7.4. The sections were incubated with this medium at 4°C in the dark overnight. After incubation, sections were rinsed for 20 min in 0.1 M PB followed by a rinse in distilled water to terminate the reaction.

Sirtuin 1 immunohistochemistry

For sirtuin 1 (SIRT1) immunohistochemistry, tissue sections were first placed in 0.01 M PBS containing 10% methanol and 3% hydrogen peroxide for 1 hr to reduce the endogenous peroxidase activity. Following this, sections were incubated in the blocking medium containing 0.1% Triton X-100, 3% normal rabbit serum and 2% bovine serum albumin (all from Sigma, St Louis, MO, USA) for 1 hr to block nonspecific binding. After several washes in PBS, the sections were incubated in goat polyclonal anti-SIRT1 (Santa Cruz sc-19857, Santa Cruz, CA, USA) antiserum at a dilution of 1:50 with the blocking medium for 48 hr at 4°C. After incubation in primary antibody, the sections were incubated with a biotinylated secondary antibody (1:200) (Vector Laboratories, Burlingame, CA, USA) at room temperature for 2 hr, followed by the standard avidin–biotin complex procedure (Vector Laboratories, Burlingame, CA, USA) with diaminobenzidine as a substrate of peroxidase.

Quantitative study and image analysis

The general approach for all quantitative image analysis was similar to our previous studies [47, 48]. The COX and SIRT1 staining was assessed in sections processed for corresponding histochemistry and immunohistochemistry. The staining intensity was quantified with a computer-based image analysis system (MGDS) along with the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). A digital camera mounted on the ZEISS microscope (Axioplane 2; Carl Zeiss MicroImaging GmbH, Hamburg, Germany) imaged sections in bright field and displayed them on a high-resolution monitor. All densitometric readings taken from all hippocampal neurons in

each image were then combined and averaged to obtain the total optical density (TOD) of each image. The background staining (BOD) of each image was measured by averaging 10 random rectangles (area of rectangle = 150 μm^2) of the lateral ventricular spaces adjoining the hippocampus. True OD for each image was then expressed by subtracting the BOD from TOD, so that each measurement was made in an unbiased way to correct for background. As the actual amount of reaction product deposited in a tissue section as a result of enzyme activity is influenced by a variety of factors, all parameters were carefully controlled following the recommended procedures for gray level adjustment, histogram stretch and minimal OD [49].

Control experiments

For control experiment of COX histochemistry, sections were incubated in the reaction medium without substrate cytochrome c. Sections in the reaction medium without primary antibody were used as control experiment for SIRT1 immunohistochemistry. In these control incubations, no histochemical or immunohistochemical labeling was detected (data not shown).

Statistical analysis

All densitometric readings taken from histochemical and immunohistochemical reactivities in un-treated and TSD animals, with or without melatonin treatment, were subjected to two-way ANOVA followed by the Bonferroni post hoc test. Behavioral data acquired from latencies of repeated trials among all experimental groups, with or without melatonin treatment, were subjected to two-way repeated measure ANOVA with Bonferroni post hoc test [50]. In all conditions, the difference was considered significant if $P < 0.05$.

Results

In normal untreated rats, numerous neurons in hippocampal formation were darkly stained with SIRT1 immunohistochemistry (Fig. 1A). The SIRT1-immunoreactive neurons were predominantly distributed in pyramidal layer of the hippocampus and granular layer of the dentate gyrus (Fig. 1A). Similar findings were also detected in TSC rats in which a moderate to strong SIRT1 staining was observed in pyramidal and granular cell layers. However, following 5 days of TSD, the SIRT1 immunoreactivity was drastically decreased (Fig. 1B). Quantitative analysis revealed that the optical density of hippocampal SIRT1 staining was significantly reduced from 1.49 ± 0.08 in normal untreated rats to 0.40 ± 0.15 in TSD ones (Fig. 3A). Comparable results were further detected in COX staining in which a remarkable decrease of hippocampal COX reactivity was observed following 5 days of TSD (1.11 ± 0.03 versus 0.31 ± 0.05 as measured in normal untreated rats and TSD ones, respectively) (Figs 2A,B and 3B). The physiological significance of reduced hippocampal SIRT1 and COX expression was demonstrated by impaired performance in spatial learning and memory. Morris water maze analysis

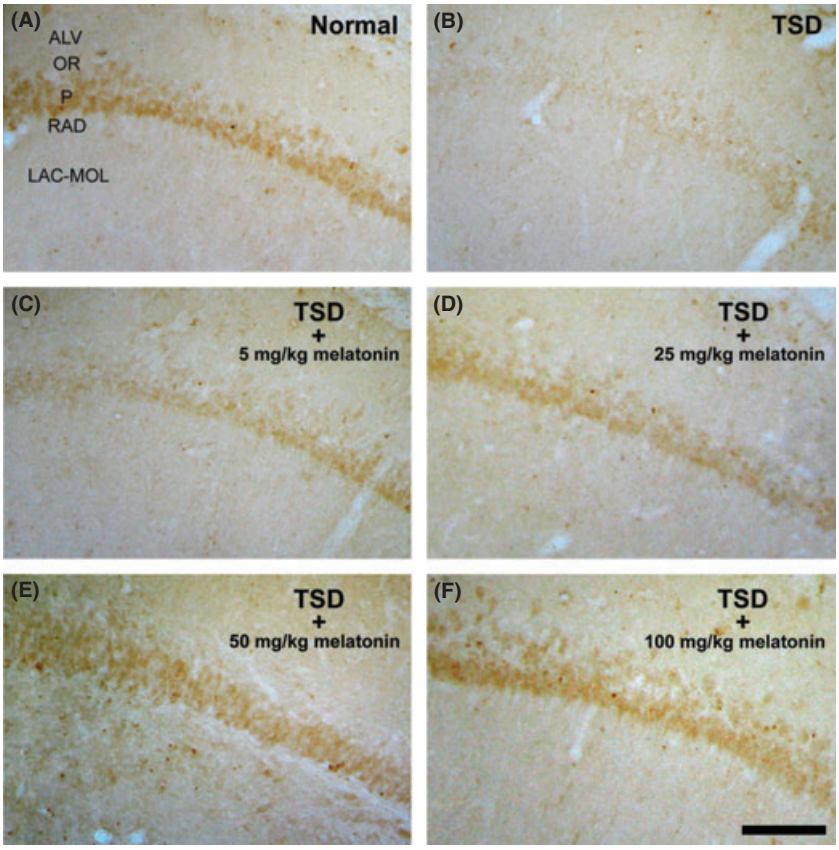

Fig. 1. Light photomicrographs showing hippocampal sirtuin (SIRT1) immunoreactivity in normal untreated (A), total sleep deprived (TSD) (B), and total sleep deprived with different doses of melatonin treated rats (C–F). Note that in normal untreated rats, numerous SIRT1 immunoreactive neurons were observed in hippocampal pyramidal cell layers (A). However, following 5 days of TSD, the SIRT1 immunoreactivity was drastically decreased (B). Also note that in rats receiving different doses of melatonin, the expression of SIRT1 was gradually returned to nearly normal levels (C–F). ALV: alveus; OR: stratum oriens; P: pyramidal cell layer; RAD: stratum radiatum; LAC-MOL: stratum lacunosum-moleculare. Scale bar = 100 μm.

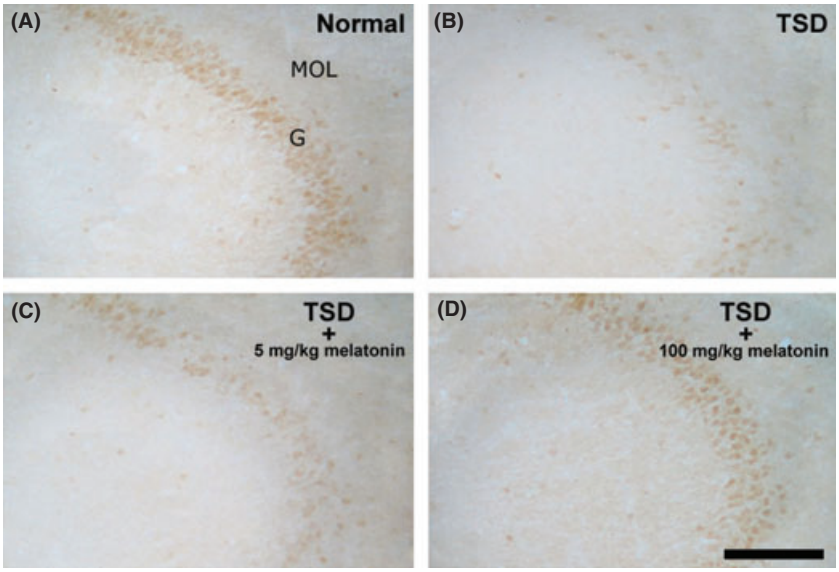

Fig. 2. Light photomicrographs showing cytochrome oxidase (COX) reactivity in dentate gyrus of normal untreated (A), total sleep deprived (TSD) (B), and total sleep deprived with 5 mg/kg (C) or 100 mg/kg melatonin-treated rats (D). Note that in normal untreated rats, numerous COX reactive neurons were observed in granular cell layers (A). However, following 5 days of TSD, the COX expression was significantly decreased (B). The reduced COX expression was increasingly returned to nearly normal levels after different doses of melatonin (C, D). MOL: molecular layer; G: granular cell layer. Scale bar = 100 μm.

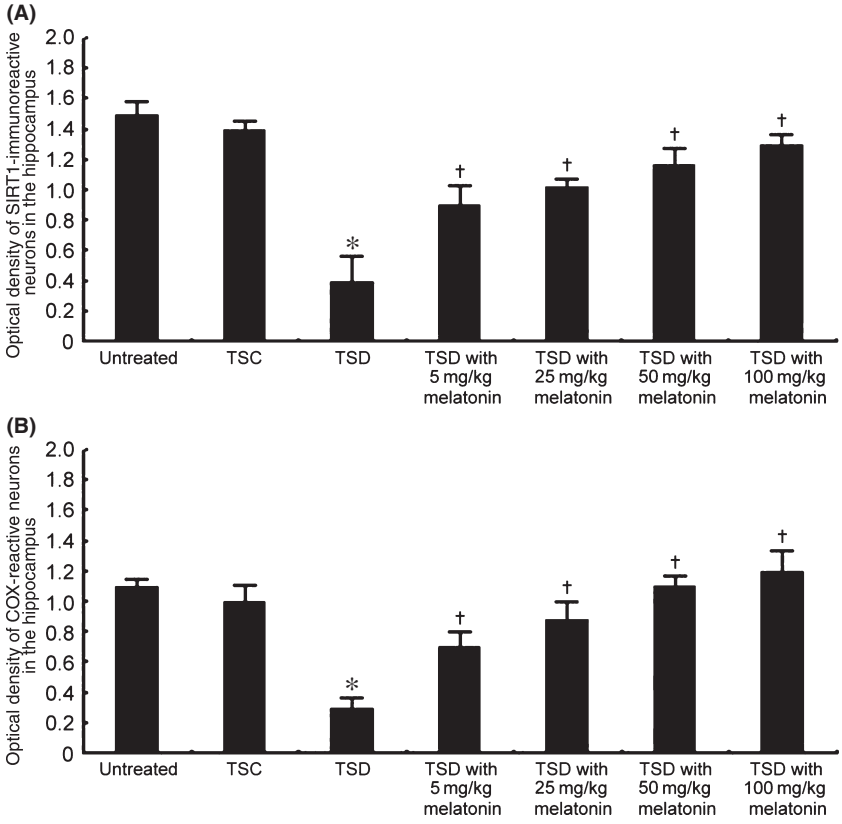

Fig. 3. Histograms showing the optical density (OD) of SIRT1 immunoreactive (A) and cytochrome oxidase (COX) reactive neurons (B) in the hippocampus of normal untreated, control for total sleep deprived (TSC), total sleep deprived (TSD), and total sleep deprived with different doses of melatonin treated rats. Note that in TSD rats, both the SIRT1 and COX staining were drastically decreased. Also note that in rats received different doses of melatonin, the OD of both SIRT1 and COX were successfully increased in a dose-dependent manner. *$P < 0.05$ as compared with normal untreated values; †$P < 0.05$ as compared with TSD values.

revealed that the average time to find the hidden platform (escape latency) in eight trials was considerably increased in TSD rats (47 ± 5.62 s) when compared with that of normal untreated animals (35 ± 2.51 s) (Fig. 4A). Data from spatial memory testing paralleled these current findings wherein TSD rats spend distinctly less percentage of time in target quadrant (19 ± 0.83%) than did the normal untreated rats (38 ± 1.25%) (Fig. 4B). It is worthy to note that one might suspect that impaired performance of TSD rats in water maze may be a direct effect of sensorimotor deficits (e.g. poor swimming motor activity) induced by TSD. Seeing that the mean latency times for animals to reach a visible platform were nearly identical between TSD and normal untreated rats (data not shown), it is reasonable to suggest that the impaired performance following TSD was definitely a spatial learning deficit resulting from neuro-plastic dysfunction, rather than caused by misgiven sensorimotor disability.

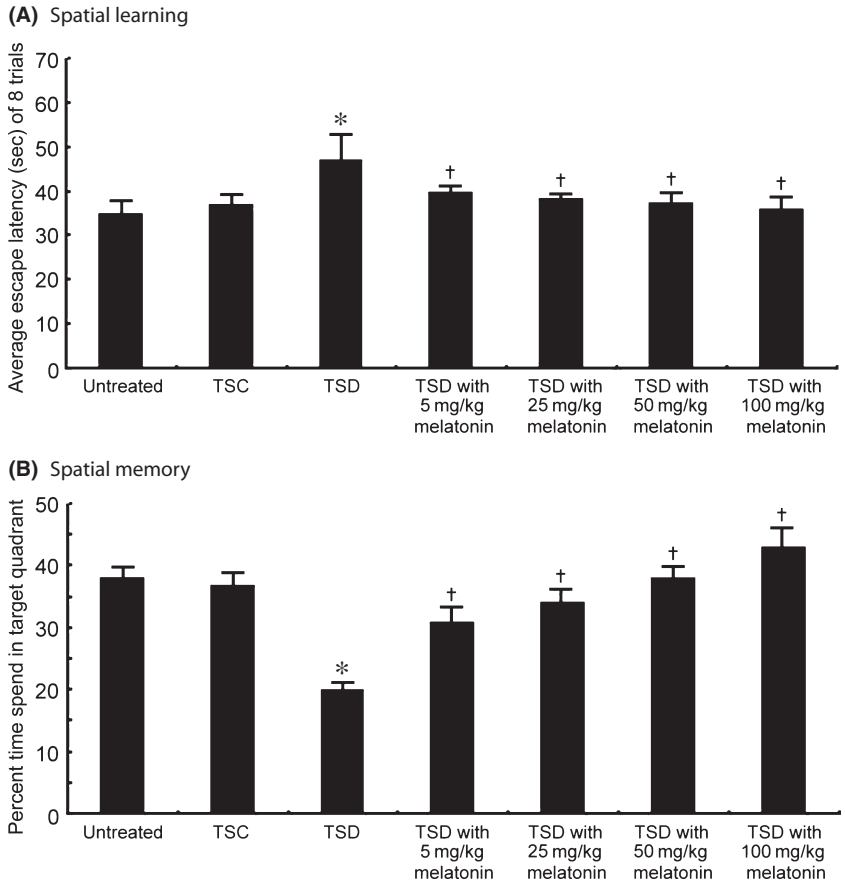

Fig. 4. Histograms showing the behavioral performance of spatial learning (A) and spatial memory (B) in Morris water maze test of normal untreated, control for total sleep deprived (TSC), total sleep deprived (TSD), and total sleep deprived with different doses of melatonin treated rats. Note that there was a considerable impairment of spatial learning and memory in rats subjected to TSD. Also note that after given different doses of melatonin, both the average escape latency and percent time spend in target quadrant were dose-dependently improved. $*P < 0.05$ as compared with normal values; $\dagger P < 0.05$ as compared with TSD values.

However, in animals receiving different doses of melatonin, the expressions of both hippocampal SIRT1 and COX were significantly preserved (Figs 1C–F and 2C,D). The preserved effects of melatonin were evident in both labeling frequency and staining intensity. Densitometric analysis revealed that the optical density of SIRT1 staining was gradually recovered from 0.40 ± 0.15 in TSD rats to 0.92 ± 0.12 in 5 mg/kg melatonin-treated ones as well as 1.36 ± 0.05 in 100 mg/kg melatonin treated group (Fig. 3A). Quantitative measurement of COX staining showed comparative results with SIRT1 reactivity in which the staining intensity of COX was progressively increased from 0.31 ± 0.05 in TSD rats to 0.72 ± 0.08 as well as to 1.21 ± 0.13 in 5 and 100 mg/kg melatonin-treated groups, respectively (Fig. 3B). The preserved activities of hippocampal SIRT1 and COX corresponded well with the improvement in behavioral performance in spatial learning and memory. This indicates that in rats receiving 100 mg/kg melatonin, the average time to find the hidden platform was significantly shorter (36 ± 2.50 s) than that of TSD ones (47 ± 5.62 s) (Fig. 4A). Results of spatial memory test also documented the neuroprotective effects of melatonin wherein the percent time spend in target quadrant was evidently increased in rats given 5 mg/kg (31 ± 2.08) or 100 mg/kg melatonin (43 ± 2.58 versus 19 ± 0.83 in TSD rats) (Fig. 4B). In addition, it is noteworthy that the advantageous effects of melatonin were dose-dependent as the maximum changes of all examined parameters were exhibited in rats receiving higher dose of melatonin (Figs 3 and 4).

As regard to stress hormone assay, neither the plasma level of corticosterone (Fig. 5A) nor ACTH (Fig. 5B) revealed noticeable differences among normal untreated, TSC, and TSD groups. The concentration of plasma corticosterone and ACTH levels measured at the end of experiment were recorded to be 48 ± 2.88 ng/mL and 55 ± 0.71 pg/mL in normal untreated rats as well as 51 ± 7.29 ng/mL and 54 ± 6.43 pg/mL in TSD ones (Fig. 5). It is noted that although the TSD rats performed by the DOW method were concurrently received larger amounts of physical activity than that of normal ones, there was no obvious increase in stress markers that were suggested to be induced by physiological loads. This means that the impaired cognitive activity observed in TSD rats solely arose from lack of sleep, and was unlikely to be attributed to other staminal factors.

In addition, in view of the close relationship between calorie availability and SIRT1-mediated metabolic regulation, two dietary parameters (the amount of food intake and the potential body weight changes) in experimental animals were further assessed in this study. The results were quantified and summarized in Table 1.

Discussion

This study provides the first functional anatomical evidence that melatonin treatment effectively preserves the relative protein levels of SIRT1 in hippocampus of total sleep-deprived rats. The preservative effect of melatonin is

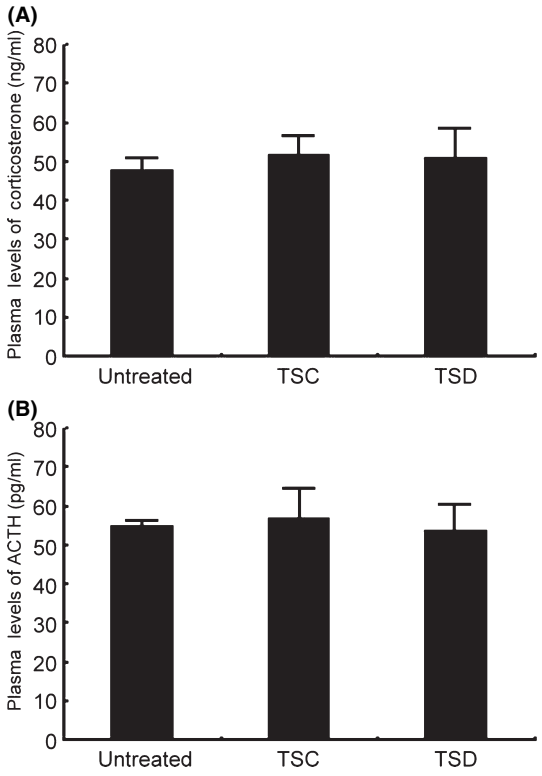

Fig. 5. Histograms showing the plasma corticosterone and ACTH levels in normal untreated, control for total sleep deprived (TSC), and total sleep deprived rats (TSD). Note that no noticeable difference in both stress markers was detected among three experimental groups.

dose-dependent as the changes were more significant in rats given higher dose of melatonin (Figs 1 and 3A). This indicates that SIRT1 is the mammalian homologue of yeast silent information regulator Sir-2, a member of sirtuin family that has recently gained much attention as a mediator of lifespan extension, metabolic homeostasis, and neuronal plasticity in several model organisms [15–20, 24–27]. Previous studies have reported that deletion of SIRT1 gene causes developmental and neuronal deficits in embryonic mice [51, 52]. Pharmacological reports also demonstrate that the inhibition of SIRT1 decreases the neuronal viability against ischemia and reperfusion injuries [53]. It is suggested that reduction of SIRT1 attenuates the neuroprotective effects via inactivation of SIRT1-mediated transcriptional profiles that affect neuronal survival and metabolic homeostasis [18, 22, 23, 27]. By acting at the interface between energy metabolism and neuronal plasticity, sufficient activation of hippocampal SIRT1 would be of great importance for maintaining the neuro-behavioral function that is essential for producing normal cognitive activities [21]. With regard to this viewpoint, significantly preserved the relative protein levels of SIRT1 in hippocampus of TSD rats following melatonin administration, as observed in this study (Figs 1 and 3A), clearly suggest that melatonin can exert its beneficial effects on cognitive function through successfully repairing the TSD-reduced hippocampal SIRT1 activity. The advanced effect of melatonin is not only seen in regulation of SIRT1, but also evident in increasing the hippocampal COX reactivity following TSD (Figs 2 and 3B). It has already been reported that melatonin-induced COX expression is positive for cell survival and neuronal plasticity [47, 54]. Through concomitantly enhancing the hippocampal COX and SIRT1 expression, melatonin may be a powerful agent in improving the metabolic status and neuronal plasticity critical for modulating learning and memory. However, it is worthy to note that SD itself has long been documented to cause conformational changes of structural proteins in the anterior brainstem [55]. Seeing as our current findings of protein expression are mainly based on immunohistochemistry, the possibilities of reduced antigenicity because of epitope masking following TSD should not be disregarded. Nevertheless, in the current study, we further detected an improved memory performance in melatonin-treated rats after TSD (Fig. 4). Given that the impaired neuronal plasticity and energy metabolism regulated by SIRT1 might serve as the underlying molecular basis responsible for cellular dysfunction [19, 56], effectively preserve hippocampal SIRT1 and its related pro-survival pathways by melatonin may thus be a potential and useful way to offset TSD-related cognitive disability.

However, although the neuroprotective function of melatonin on preserving the SIRT1 has been well documented, other mechanisms by which melatonin could exert its powerful actions on cognitive activity should not be overlooked. Previous studies have indicated that melatonin directly interacts with both excitatory and inhibitory neurotransmitter systems that might alter the synaptic transmission between hippocampal neurons [33, 34]. Electrophysiological studies also reported that melatonin modulates the intrinsic excitability of hippocampal neurons and participates in the regulation of memory, cognition as well as emotions [57, 58]. It is suggested that melatonin may exert its effects on the hippocampus through binding to two G-protein coupled membrane receptors (MT_1 and MT_2)

Table 1. Whole body weight (g) and number of chow pill eating of normal untreated, control for total sleep deprived (TSC), and total sleep-deprived rats (TSD) with or without melatonin treatment (measured at the end of experiment)

	Experimental Group				
	Normal untreated	TSC	TSD	TSD with 5 mg/kg melatonin	TSD with 100 mg/kg melatonin
Whole body weight (g)	243 ± 11	236 ± 18	220 ± 15	227 ± 13	229 ± 17
Number of chow pill eating	12 ± 3	15 ± 2	21 ± 4*	17 ± 2	18 ± 5

Data are expressed as mean ± S.D. *$P < 0.05$ as compared with normal untreated values.

[59]. By receptor-mediated induction of continuous changes in intracellular signaling pathways, melatonin could modify the firing status (either facilitation or depression) of hippocampal neurons, and therefore produce plastic changes that underlie the processing of cognitive activity [57, 59, 60]. Melatonin application has long been shown to yield a permanent facilitatory effect on short-term memory [61]. Furthermore, in addition to regulating the temporal structure of hippocampal circuits involved in learning and memory, melatonin has also been demonstrated to interact with calmodulin (CaM) and protein kinase C (PKC), which plays an important role in cytoskeletal organization and neurite outgrowth in developing neurons [36]. It is well established that dynamic changes in cytoskeletal organization during neuritogenesis is crucial for neurons to constitute a specific pattern of synaptic connectivity [62]. Seeing that melatonin modulates the initial stages of neurodevelopment and restoring the cytoskeletal assembly [36], application of melatonin will be of great help in the treatment of cognitive dysfunction wherein cytoskeletal de-arrangement and neuro-plastic abnormality is a participant. In addition, it is noteworthy that melatonin and its metabolites also exert beneficial functions via their anti-oxidative activity [28, 29, 48, 63]. It is reported that increased hippocampal oxidative stress also plays an important role in cognitive impairment caused by TSD [9]. As treatment with melatonin improves the cognitive function following TSD (Fig. 4), this study suggests that the neuroprotective effects of melatonin, possibly along with its metabolites, may also be related to its significant anti-oxidative activity.

On the other hand, it is worthy to note that as SIRT1 is a metabolic switch protein that links the cellular state with energy metabolism [15, 16]; thus, the possibility that any dietary effect on SIRT1 should not be discarded. During the past few years, many studies have demonstrated that calorie restriction (CR) is a dietary regimen directly responsible for enhancing the SIRT1 activity [17, 25]. Seeing that TSD has been reported to cause weight loss in experimental animals [64], one might expect that TSD may serve as a practical way to augment the SIRT1 activity. This seems to contradict our current findings in which a reduced SIRT1 expression was detected following TSD. However, in view of the fact that decreased body weight does not reliably mean the extent of calorie availability [65], the amount of food intake during TSD should be further assessed to clarify the relationship between dietary issue and SIRT1 activity. The present results indicated that in normal untreated rats, the average number of chow pill eaten was 12 ± 3 (Table 1). However, following TSD, the consumption of chow pills was significantly increased to 21 ± 4 (Table 1). Although the detail mechanisms concerning the interaction between weight loss and overeating are still unresolved, this finding was in good agreement with previous study in which an increment of food intake was clearly demonstrated in rats subjected to TSD [66]. It has been demonstrated that high calorie feeding depresses the hippocampal SIRT1 activity [21]. With regard to these viewpoints, it is reasonable to suggest that because of hyperphagic response (and its related calorie ingestion), TSD would exert a negative effect on SIRT1 instead of exaggerating its activity by restricted conditioning as misrecognized previously.

Another important issue to be addressed is that TSD itself has long been considered to be stressful for experimental animals [41]. Restricted sleep would not only cause neurobehavioral deficits, but also yield neuro-chemical and neuro-metabolic changes (Figs 1A,B and 2A,B) that ultimately lead to excessive allostatic stress load which consequently disrupts brain plasticity [5, 10]. A variety of studies have demonstrated that either physiological or psychological stress alone may be a significant cause for memory impairment without other experimental insults [67–69]. Considering that our experimental paradigm was performed by the DOW method, the possibility that rats subjected to TSD were simultaneously suffered from physiological loads (e.g. walking against the disc rotation) that might consequently lead to memory deficits should be carefully considered. In this case, this study also measured the plasma levels of stress hormone corticosterone and ACTH to further elucidate whether physical activity accompanied TSD would produce any obvious impact on memory. The results indicated that neither corticosterone nor ACTH levels showed noticeable differences among normal untreated, TSC and TSD groups (Fig. 5). Metabolic, neurochemical, as well as behavioral data also showed comparable results between TSC and normal untreated animals (Figs 3 and 4), even though the former have received large amount of physical activity than that of normal ones. It is suggested that the current physical activity, together with its related physiological loads, did not exert significant depressing effect on memory performance across 5 days of TSD. Based on this viewpoint, it is rational to suggest that the memory impairment observed following TSD, at least in this study, is a specific effect of sleep loss and not likely to be attributed to other confounding factors. However, as prolonged physical debilitation has already been regarded as a chronic stressor [70, 71], it should not preclude the possibilities that stress hormone level and other forms of neuro-cognitive task might indeed be impaired by using the DOW model with more extended TSD period.

In summary, this study has addressed for the first time whether melatonin treatment would successfully preserve hippocampal SIRT1 activity in adult rats subjected to TSD. The positive function of melatonin is probably expressed by enhancing the SIRT1-mediated metabolic regulation and neuronal plasticity that contributes to normal cognitive activity. Although the detailed cellular and molecular mechanisms related to TSD-induced cognitive impairment still remains unclear, the powerful neuroprotective effects of melatonin may thus serve as an attractive candidate and novel strategy for clinical treatment of cognitive dysfunction resulted from increasing prevalence of sleep deficiency.

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一、參加會議經過與心得

承蒙行政院國家科學委員會於經費上之鼎力支持，職有幸參加西元二００九年第十九屆歐洲微生物學暨感染症國際會議。本屆會議於今年五月十六日至五月二十日於芬蘭首都赫爾辛基市舉行。歐洲微生物學暨感染症學會為一整合歐洲各國微生物學與感染症研究之學術組織，每年固定召開一次國際性學術會議，每次會議皆吸引相當多世界各地微生物學與感染症研究學者熱烈參與，與會者除了可在議程中，汲取微生物學與感染症研究發展的最新進展外，更可透過論文發表及展示，與世界各地之學者專家互相討論及分析，藉由彼此間的腦力激盪，不但可拓展自身的學術視野，更有助於學術交流的進行。職此次獲國科會各級長官的大力支持，代表中山醫學大學醫學系解剖學科，公差假赴芬蘭參加本屆之歐洲微生物學暨感染症國際會議，並在大會中發表學術論文，除增進自身之研究深度與廣度外，並積極與世界各國學者專家做學術交流，希望對提升本國暨服務單位之國際學術形象暨知名度有所助益。

職服務於中山醫學大學期間，曾多次代表解剖學科出席國際上與基礎暨臨床醫學研究相關之學術會議，包括近年內赴德國柏林參加第一屆世界睡眠醫學大會、赴俄羅斯聖彼得堡參加第十一屆國際壓力與行為神經科學會議，並於會中發表學術論文，認識並結交不少國外學者。本次參加之第十九屆歐洲微生物學暨感染症國際會議由芬蘭主辦，議期為期五天，在芬蘭首都 — 有「波羅的海女兒」之稱的赫爾辛基市盛大舉行。此次會議共吸引了來自世界各地約六百位專家學者共襄盛舉，全球研究微生物學與感染症之基礎與臨床專家藉此機會齊聚一堂，除了可交換彼此的研究心得與經驗外，更可透過相互觀摩與討論的機會，達到拓展自身研究視野與增進良性學術交流的目的。

本屆會議探討之學術主題相當廣泛，大致上可分為愛滋病防範 (HIV prevention)、抗生素使用 (antibiotic usage)、細菌分子生物學 (molecular biology of bacterium)、病毒變異與傳播 (viral variation and spread)、新興傳染病之診斷與預防 (prevention and diagnosis of newly infectious diseases)、痢疾與相關旅遊疾病 (malaria and related travel diseases)、細菌性腦膜腦炎 (bacterial meningoencephalitis)、院內感染 (nosocomial infection)、肺炎 (pneumonia)、病毒性肝炎 (viral hepatitis)、肺結核 (tuberculosis)、外科手術感染 (surgical infection)、黴菌感染 (fugal infection) 與菌血症 (sepsis) 等數個專區，每個專區皆安排來自全球各領域的大師精闢演講，並開放壁報陳列與展示，讓對某一特定主題有興趣的研究學者充分參與，除享受浸淫於涉獵新知的喜悅外，並充分感受到學術發展與醫療進步的濃厚氣息。

值得一提的是，大會於議程進行中每天早上十一點半至十二點半，及每天下午五點

半至六點半，各皆安排數場世界上著名的感染症研究學者做專題演講，講題分別為：

1. Reflections of infectious diseases in music
2. FUN-gi: thoughts on yeasts, moulds and medicine
3. The bacterial pathogen Listeria monocytogenes: a multi-faceted model
4. The potential role of metagenomics in clinical infectious diseases
5. Update on the H1N1 influenza A outbreak
6. Challenges for a new infectious diseases institute in Africa
7. Infections and the Guillain-Barré syndrome
8. Hantavirus infections in Europe
9. Improving quality of care and patient safety in ICU
10. Pneumococci and the host

每場演講內容皆深入簡出，研究設計切入核心，聆聽大師精闢的演講，往往令人有收穫豐厚、不虛此行之感慨。於專題演講後，大會接續安排了半小時的休息時間，會議主辦單位提供了精緻的小點心與咖啡供各與會學者食用及品嚐，於此同時，與會學者亦可自由至壁報展示區瀏覽參展論文，並可駐足於有興趣的論文看板前，與論文作者做進一步的交流及討論。口頭論文的報告則安排於每日早上九點至十一點，及每日下午四點至六點等兩個時段舉行。由於口頭論文的發表場地不只一處，同一時段通常有三至五個論文同時舉行，與會學者必須選擇最有興趣的一個主題前往聆聽，每一論文的報告時間為十五分鐘，接著由聽眾發表問題，最後再由論文作者逐一解答。職於參加大會口頭論文發表的過程中，不但親自見識到國外學者專家治學之嚴謹、研究思路之清晰、邏輯推理之正確，更重要的，是其發表問題之深入與回答問題之技巧，這些所得豐厚之見聞，是參加此一大型國際學術會議的重要收穫。此外，藉由瀏覽參展論文與聆聽口頭報告的機會中，職亦觀賞到了各國論文發表學者精美的海報版面與提綱契領的幻燈片製作，透過觀摩學習這些論文發表方面的實用技巧，對將來個人學術研發方面的成果展示上，亦是相當難能可貴的經驗。職於本屆大會中，用壁報展示的方式，發表了一篇學術論文。論文題目為：Are CTX-M beta-lactamases associated with poorer clinical outcomes in bloodstream infections caused by ESBL-producing E. coli？論文主旨旨在探討患有 CTX-M ESBL 抗藥性基因之大腸桿菌菌血症病人與患有非 CTX-M ESBL 抗藥性基因之大腸桿菌菌血症病人於臨床上是否有較差之預後表現。我們的論文陳列吸引了多數學者專家到場觀賞。期間亦有不少專家學者提出疑問或批評，職都一一詳細回答，對於建設性的討論與指教，我們更是銘記於心，並希望於回國服務後，做更進一步的研究或探

討，將參與會議之所見所聞做詳實的整理與記錄，為感染症之研究貢獻一己之力量。

二、建議

參與國際醫學會議為醫學研究及醫療服務人員教育訓練的重要一環，在現今學術研究競爭激烈的環境中，身為基礎醫學研究領域的一份子，每個人皆應擁有強烈的使命感與宏偉的世界觀。當今政府正積極推展務實外交，要讓台灣的產業界及學術界與世界接軌，值此各方面皆蓬勃發展、且知識爆炸的時刻，職誠摯建議所有出席國際醫學會議的與會代表，皆能秉持為國家、為學校增光的原則，善加利用、把握此一難得的學習與外交機會，除了豐厚自己的學術識養外，尚能為學校與國家整體學術地位之提升，貢獻自身的一點心力！