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睡眠剝奪與呼吸中止對大鼠前額葉皮質相關神經化學物質活性表現與功能狀態改變之影響

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GENDER DIFFERENCES OF CYTOCHROME OXIDASE REACTIVITY IN THE HIPPOCAMPUS OF TOTAL SLEEP DEPRIVED RATS

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Sleep disorders are a common cause of neurocognitive impairment that has been implicated as a risk factor for neuropsychological disease. While both epidemiological and clinic-based studies indicate that neuropsychological diseases are more common in women than in men, the present study is aimed to determine whether there is any gender difference in the functional status of hippocampal neurons following severe sleep disturbance. Sleep disturbance was achieved by subjecting the adult rats to fives days of total sleep deprivation (TSD). The cytochrome oxidase (COX) histochemistry was used to detect the functional status of the hippocampus. The results indicated that in normal rats, enhanced COX reactivity was detected in the entire region of hippocampus without gender difference. However, following TSD, the staining intensity of COX reactivity was drastically decreased in the hippocampus of both genders with a more significant dropping in females. Since COX reactivity could serve as an endogenous metabolic marker for neuronal activity, the remarkable decreasing of COX reactivity following TSD may imply that TSD will suppress the neuronal activity of hippocampal neurons. The pronounced reduction of COX expression observed in female rats than in male rats further suggests that TSD would exert a worsened effect on functional status of females. These neurochemical findings might thus provide new insights into the pathophysiological mechanisms underpinning the gender difference susceptibility of sleep-related neuropsychological diseases.

INTRODUCTION

It is well established that sleep plays an important role in neurobiological, physiological and behavioral processes. Prolonged sleep loss or frequently disrupted of sleep can result in mental changes and impaired cognitive ability, which has been suggested to serve as a risk factor for the development of major neuropsychological diseases. Several lines of epidemiological and clinic-based evidence have indicated that the prevalence of neuropsychological illness is twice as frequently in females than in males. These findings would thus likely lead us to propose a potential possibility that there may possess some sexual dimorphisms in the neuronal regions involving in the integration of cognitive functions.

It has been known that the hippocampal formation is one of the critical regions responsible for the mediation of neuronal circuits relevant to cognitive function. This structure receives inputs from diverse areas of the neuraxis and participates in complicated and interrelated behaviors such as memory, learning and social interactions. Previous reports have indicated that sleep deprivation would cause behavioral, synaptic, and membrane excitability alterations in hippocampal neurons, which could ultimately decrease the long-term potentiation (LTP) capacity. Biochemical studies also demonstrated that diminished nitric oxide synthase (NOS) reactivity, the key molecular mechanism underlying LTP induction, would reduce the firing properties of the hippocampal principal cells and subsequently contribute to sleep deprivation-induced synaptic deficiency and memory impairments. It is indicated that the neuronal activity is closely correlated to its metabolic stage. The variations of neuronal activity could be reflected by corresponding differences in the levels of energy metabolism. Since the cytochrome oxidase (COX) reactivity is a reliable marker for neuronal metabolic stage, the intensity of COX staining within the soma may be related to the frequency of a neuron’s spontaneous spiking or synaptically-evoked activity.

However, although the detrimental effects of sleep deprivation on hippocampal formation has been greatly reported at the molecular, cellular or biochemical levels, the anatomical profiles of the possible gender difference of this region responding to sleep deprivation is still poorly understood. Moreover, whether the mechanism responsible for the occurrence of gender difference in sleepless-induced neuropsychological vulnerability is resulted from the metabolic dysfunction within the hippocampal formation is remained to be explored. With regard to this viewpoint, the aim of the present study is to investigate the

possibility by verifying the neuronal COX immuno-expression in the hippocampal formation of both genders subjecting to severe sleep deprivation treatment. In addition, in order to examine whether the metabolic alterations would affect the LTP activation, the neuronal NOS reactivity in the hippocampal formation is further quantitatively evaluated.

MATERIALS AND METHODS

Treatments of experimental animals

Adult male and female Wistar rats (n = 36, with 18 in each gender, weighing 200 ~ 250 g) were used in this study. The experimental animals were divided equally into six groups. Animals in the first two groups were composed of male and female rats, respectively, and subjected to TSD for five days (TSD-m and TSD-f group), while those in the third and fourth groups were housed in the TSD apparatus but were permitted to sleep (control for sleep deprivation, TSC-m and TSC-f group). For animals in the last two groups, they were kept in the plastic cage placed aside from the TSD apparatus and served as normal untreated controls (Untreated-m and Untreated-f group). TSD was performed by the disc-on-water (DOW) method modified after that of Chang (Chang et al., 2006). Briefly, the apparatus was composed of two rectangular clear plastic chambers placing side by side. A single plastic disc serving as the rat-carrying platform was built in the lower quarter of the two chambers. Beneath the disc and extending to the chamber walls was a rectangular tray that was filled with water to a depth of 5 cm. An electric motor was set to run the rat-carrying disc at a moderate speed of 3.5 rev/min for 5 days; it was considered as a complete cycle for 8 s with an interval for 15 s. Sleep deprivation depends on the rat's aversion to water, since rats rarely entered the water spontaneously. As sleep deprivation begins, rats in the TSD groups placing on the disc had to keep awake and walk against the direction of disc rotation to avoid being forced into the water. For TSC groups, rats were received the same physical activity as that of the TSD groups except that they were allowed to sleep from 06:00 ~ 18:00 wherein no disc movement was initiated. All experimental animals were exposed to an automatically regulated light:dark cycle of 12:12 at a constant temperature of 25℃ ± 1℃. Food and water were made available through grids placed on top of the chambers.

Perfusion and tissue preparation

For quantitative immunohistochemical studies, rats of all experimental groups were deeply anesthetized with 7% chloral hydrate and perfused transcardially with 0.9 % saline

followed by 300 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The hippocampal formation was then removed and post-fixed in the same fixative for 2 h, followed by overnight immersion in 30 % sucrose buffer at 4℃ for cryoprotection. Serial 30 μm thick sections of the hippocampus were cut transversely with a cryostat on the following day and were alternatively placed into two wells of a cell culture plate. Sections collected in the first well were processed for COX immunohistochemistry, and those in the second well were processed for NADPH-d (a co-factor of NOS) histochemistry.

COX immunohistochemistry

For COX immunohistochemistry, tissue sections collected in the first well were first placed in 0.01M phosphate buffer saline (PBS), pH 7.4, containing 10% methanol and 3% hydrogen peroxide for 1 h 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 for 1 h to block nonspecific binding. After several washes in PBS, the sections were incubated in the goat polyclonal anti-COX antiserum at the dilution of 1:250 with the blocking medium for 48 h at 4℃. After the incubation in primary antibody, sections were further incubated with a biotinylated secondary antibody (1:200) at room temperature for 2 h. The reaction product was revealed by the standard avidin-biotin complex (ABC) procedure with 3,3'-diaminobenzidine as a substrate of peroxidase.

NADPH-d histochemistry

Tissue samples processed for NADPH-d histochemistry were treated in NADPH-d reaction medium containing 0.1 mg/ml nitroblue tetrazolium, 1 mg/ml β-NADPH and 0.3 % Triton X-100 in 0.1 M PB (pH 7.4) for 1 h at 37℃. After incubation, the sections were washed several times in 0.1 M PB to terminate the reaction.

Quantitative image analysis

The general approach for all quantitative image analysis was similar to our previous studies (Chang et al., 2000; 2001; 2002; 2003; 2004; 2005). The COX staining intensity was quantified with a computer based image analysis system (MGDS) and Image-Pro Plus software. A digital camera mounted on the ZEISS microscope imaged sections in bright field and displayed them on a high-resolution monitor. The optical density (OD) of reaction product in the hippocampal formation was determined using a "mouse" to draw an area encircling the subdivisions of the hippocampal region. The recorded densitometric reading

represents the optical density of the pixels comprising that area. All densitometric readings taken from all of the subdivisions in each section were then combined and averaged to obtain the total OD (TOD) of each section. The background staining (BOD) of each section was measured by averaging ten random rectangles (area of rectangle = 150 μm^2) of the corpus callosum in each section. The true OD for each section was then expressed as the value by subtracting the BOD from TOD (TOD − BOD), so that each measurement was made unbiasedly to correct for background. Comparison for the values obtained from each gender of untreated, TSC, and TSD rats were subjected to two-way ANOVA analysis. The effect of each challenge compared with untreated group was further analyzed using the Bonferroni *post hoc* test. The statistical difference was considered significant if $P<0.05$.

RESULTS

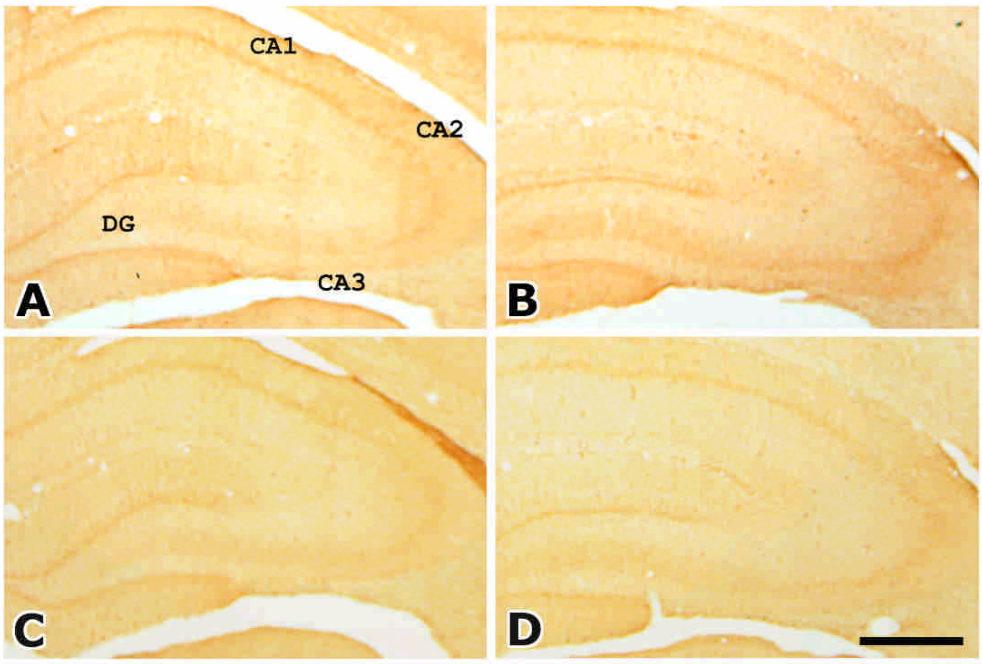

Fig. 1. Light photomicrographs showing the cytochrome oxidase (COX) reactivity in the hippocampus of normal (A,B) and total sleep-deprived rats (C,D). Note that in normal untreated rats, numerous hippocampal regions (CA1 ~ CA3) were positively stained for COX reactivity in both the male (A) and female rats (B). However, following total sleep deprivation, the COX staining was significantly decreased in the hippocampus of both genders with a prominent decreasing in the female (D) than that in male ones (C). DG: dentate gyrus, Scale bar = 200 μm.

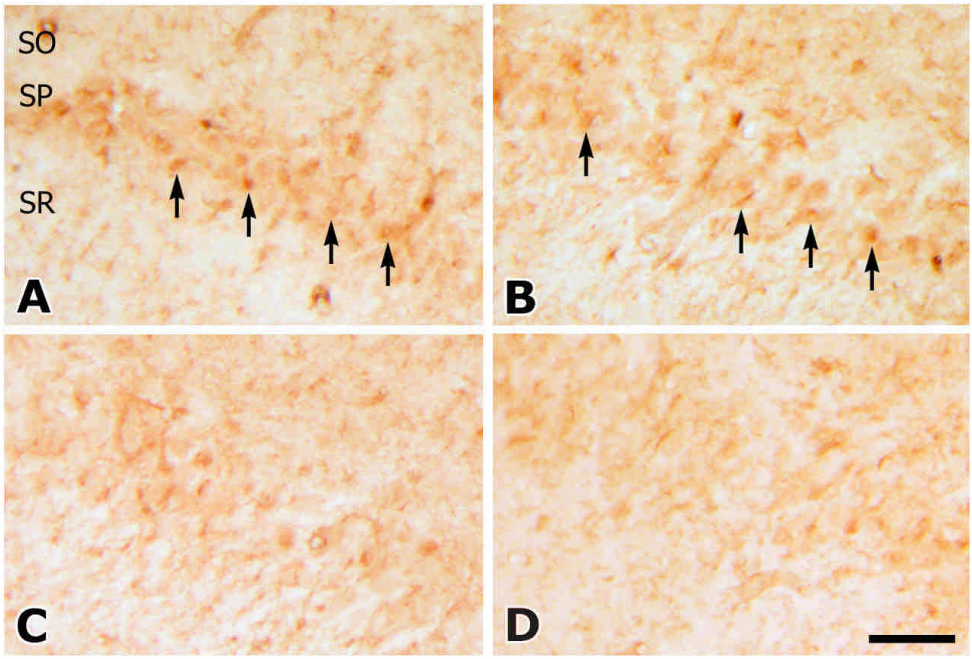

Fig. 2. Light photomicrographs showing the higher magnification of cytochrome oxidase (COX) reactivity in the CA1 region of normal (A,B) and total sleep-deprived rats (C,D). Note that in normal untreated rats, numerous COX reactive neurons with various staining intensity (arrows) were identified in the stratum pyramidale (SP) of both the male (A) and female rats (B). However, following total sleep deprivation, both the frequency and staining intensity of COX reactive neurons were drastically decreased in the SP of both genders with a prominent decreasing in the female (D) than that in male ones (C). SO: stratum oriens, SR: stratum radiatum, Scale bar = 50 μm.

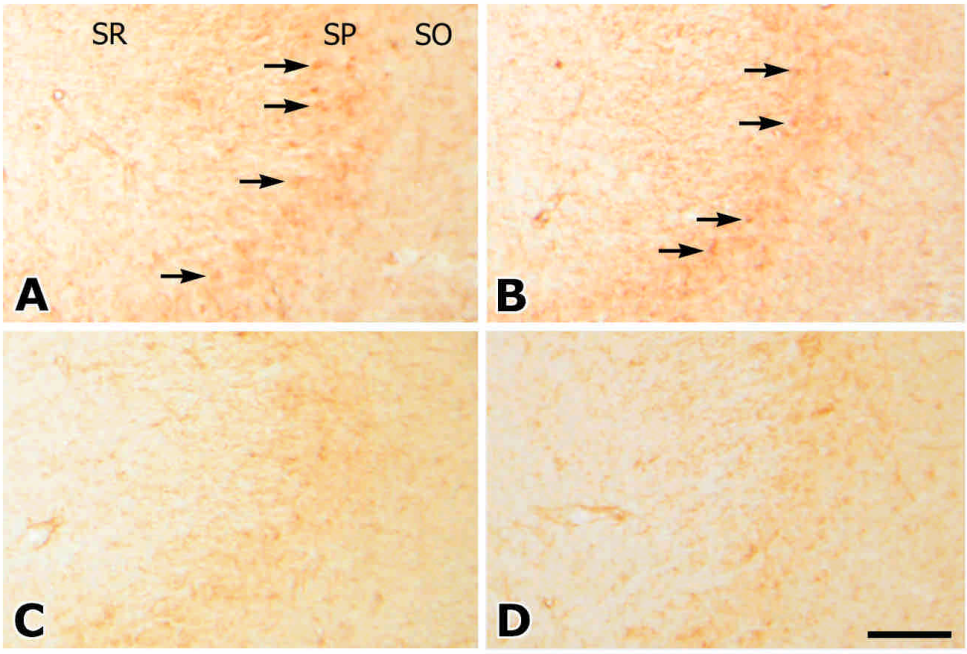

Fig. 3. Light photomicrographs showing the higher magnification of cytochrome oxidase (COX) reactivity in the CA2 region of normal (A,B) and total sleep-deprived rats (C,D). Note that in normal untreated rats, numerous COX reactive neurons with various staining intensity (arrows) were identified in the stratum pyramidale (SP) of both the male (A) and female rats (B). However, following total sleep deprivation, both the frequency and staining intensity of COX reactive neurons were drastically decreased in the SP of both genders with a prominent decreasing in the female (D) than that in male ones (C). SO: stratum oriens, SR: stratum radiatum, Scale bar = 50 μm.

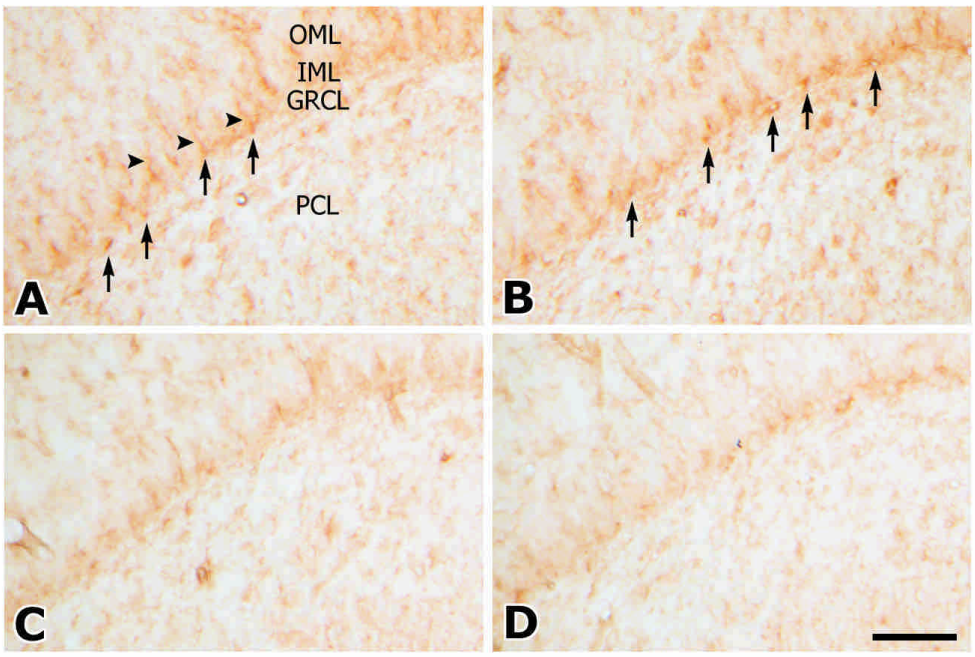

Fig. 4. Light photomicrographs showing the higher magnification of cytochrome oxidase (COX) reactivity in the dentate gyrus of normal (A,B) and total sleep-deprived rats (C,D). Note that in normal untreated rats, numerous COX reactive neurons with significant processes (arrowheads) were identified in the granular cell layer (GRCL) of both the male (A) and female rats (B). However, following total sleep deprivation, both the frequency and arborization of COX reactive neurons were drastically decreased in the GRCL of both genders with a prominent decreasing in the female (D) than that in male ones (C). OML: outer molecular layer, IML: inner molecular layer, PCL: polymorphic cell layer, Scale bar = 50 μm.

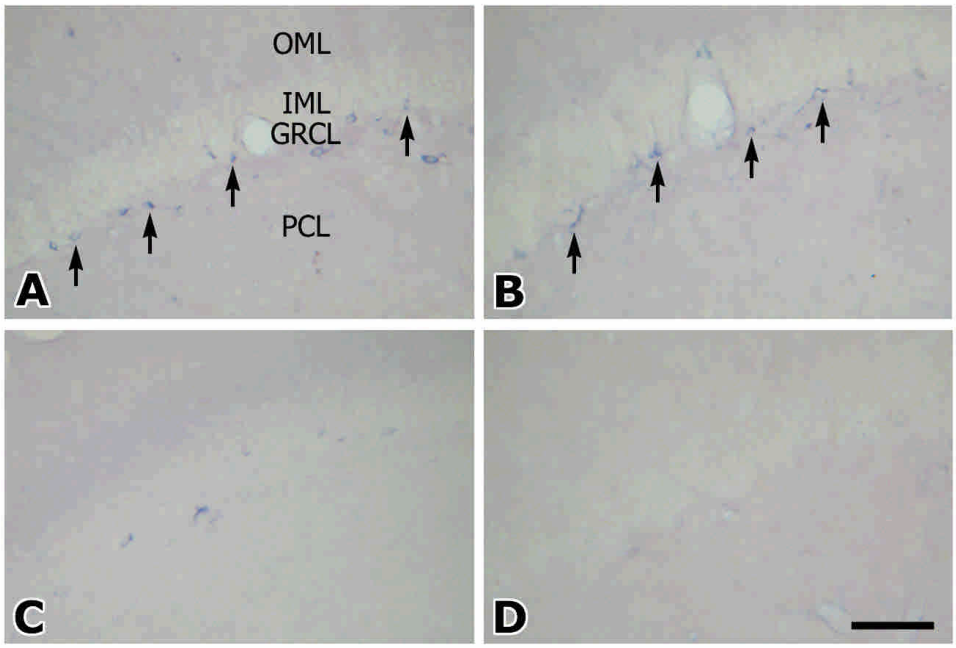

Fig. 5. Light photomicrographs showing the higher magnification of cytochrome oxidase (COX) reactivity in the dentate gyrus of normal (A,B) and total sleep-deprived rats (C,D). Note that in normal untreated rats, numerous NADPH-d reactive neurons were identified in the granular cell layer (GRCL) of both the male (A) and female rats (B). However, following total sleep deprivation, both the frequency and staining intensity of NADPH-d reactive neurons were drastically decreased in the GRCL of both genders with a prominent decreasing in the female (D) than that in male ones (C). OML: outer molecular layer, IML: inner molecular layer, PCL: polymorphic cell layer, Scale bar = 50 μm.

CONCLUSIONS

1. The present study has provided the first functional anatomical evidence the TSD would effectively suppress the COX immunoreactivity in the hippocampal formation of both adult male and female rats. The reduction of COX staining is obvious in all subfields of hippocampal formation with a more significant dropping in female rats compared with that of male ones.

2. The decrease of the COX staining following TSD may indicate that some metabolic deficiency would occur in the hippocampal neurons. The metabolic dysfunction in the hippocampal formation may serve as a potential source for the development of major cognitive disorders.

3. The more prominent reduction of COX expression observed in the female rats suggests that female ones would exhibit a higher vulnerability to TSD relevant cognitive diseases. This finding is consistent with the clinical observations that females are more prone to suffer from neuropsychological illness after a prolonged sleep disturbance.