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Frontal cortical mitochondrial dysfunction and mitochondria-related β-amyloid accumulation by chronic sleep restriction in mice

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Mitochondrial dysfunction induced by mitochondria-related β-amyloid (Aβ) accumulation is increasingly being considered a novel risk factor for sporadic Alzheimer's disease pathophysiology. The close relationship between chronic sleep restriction (CSR) and cortical Aβ elevation was confirmed recently. By assessing frontal cortical mitochondrial function (electron microscopy manifestation, cytochrome C oxidase concentration, ATP level, and mitochondrial membrane potential) and the levels of mitochondria-related Aβ in 9-month-old adult male C57BL/6J mice subjected to CSR and as an environmental control (CO) group, we aimed to evaluate the association of CSR with mitochondrial dysfunction and mitochondria-related Aβ accumulation. In this study, frontal cortical mitochondrial dysfunction was significantly more severe in CSR mice compared with CO animals. Furthermore, CSR mice showed higher mitochondria-associated Aβ, total Aβ, and mitochondria-related β-amyloid protein precursor (AβPP) levels compared with CO mice. In the CSR model, mouse frontal cortical mitochondrial dysfunction was correlated with mitochondria-associated Aβ and mitochondria-related AβPP levels. However, frontal cortical mitochondria-associated Aβ levels showed no significant association with cortical total Aβ and mitochondrial AβPP concentrations. These findings indicated that CSR-induced frontal cortical mitochondrial dysfunction and mitochondria-related Aβ accumulation, which was closely related to mitochondrial dysfunction under CSR. *NeuroReport* 27:916–922

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Introduction

Sleep deprivation caused by intense work schedules is a characteristic of the modern society. According to recent surveys, an increasing number of individuals are experiencing sleep deprivation. The National Sleep Foundation's 2003 Sleep in America poll found that more than 20% of Americans sleep less than 6 h per day [1]. Clinical findings indicated that sleep plays an integral role in metabolic control; as a result, insufficient sleep could contribute toward many pathological aspects, including weight loss [2], high energy expenditure, deterioration in physical appearance [3], and development of type 2 diabetes [4]. Meanwhile, a number of animal studies confirmed that sleep deprivation could impair the formation of long-term potentiation [5,6], alter the synthesis of extracellular signal-regulated kinases [7,8], and affect amounts of neurotransmitters [9]. Multiple studies have recently focused on the association of brain β-amyloid (Aβ) dynamics with sleep restriction both in mice and in humans [10,11].

β-Amyloid protein precursor (AβPP) is sequentially cleaved by β-site AβPP cleaving enzyme 1 and the γ-secretase proteolytic complex to produce various Aβ peptides, a cascade considered the major pathophysiological alteration in Alzheimer's disease (AD) and other neurodegenerative diseases [12]. Intracellular and extracellular Aβ accumulations cause neuronal dysfunction (especially mitochondrial dysfunction), which constitutes the mechanism underlying memory impairment and other clinical symptoms in AD patients [13,14]. In the first decade of 21st century, several researchers reported the correlation between sleep deprivation and brain mitochondrial dysfunction. In 2008, Yang *et al.* [15] reported that paradoxical sleep deprivation (PSD) induces Bax translocation into mitochondria and promotes cytochrome C release into the cytoplasm, causing deficits in learning behavior. In 2010, Andreazza *et al.* [16] attempted to characterize the function of the mitochondrial electron transport chain in the brain using an animal

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model of PSD and found a more significant decrease in complex I–III activity in the PSD group compared with the control (CO) animals. However, reports assessing whether chronic sleep restriction (CSR) is harmful to cortical mitochondrial function with the involvement of mitochondria-related Aβ are scarce. Therefore, a 2-month sleep restriction mouse model was used to explore the frontal cortical mitochondrial changes caused by CSR.

Materials and methods

Animals and housing

Nine-month-old adult male C57BL/6J mice (Slac, Shanghai, China) weighing ~28 g were housed under a 12 h light/12 h dark cycle, with room temperature maintained at 21 ± 1°C. They were provided with food and water throughout the experiment. The study was approved by the Ethics Committee of Animal Experiments of the Second Military Medical University.

Experimental design

Grouping

Sixteen C57BL/6J mice were divided into two groups of eight: CSR group and CO group.

Sleep restriction and forced activity

The sleep restriction protocol allowed the mice to sleep 4 h per day at the beginning of the light phase (08 : 00–12 : 00 h) in their home cage [17]. For the rest of the day, the animals were kept awake by placing them on slowly rotating wheels (40 cm in diameter) driven by an engine at a constant speed (0.4 m/min). As the sleep-deprivation procedure included mild forced locomotion, forced activity CO mice were used. Animals of the CO group were placed on the same plastic drums as the sleep restriction models. However, the wheels rotated at a faster speed (0.66 m/min) and for a shorter time (12 h). With this protocol, CO mice walked the same distance as sleep-restricted models, but had sufficient time for sleep (20 h). Animals were subjected to forced activity during the dark phase, which is their circadian activity phase. Before the experiments, mice were habituated to the apparatus by placing them on the wheels for 1 h over 3 days. After the sleep-restriction procedure, all the mice were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and decapitated. The brains were immediately removed for the following experiments.

Isolation of cortical mitochondria

Mitochondria samples were prepared using a commercially available mitochondria isolation kit (Beyotime, Haimen, China) according to the manufacturer's instructions. Approximately 100 mg of frontal cortical tissue was homogenized in 1.5 µl of homogenization medium containing the following reagents: 70 mM sucrose, 210 mM mannitol, 2 mM HEPES, and 0.1 mM EDTA. The homogenate was centrifuged at 600*g* for 5 min and the resultant supernatant was centrifuged at 11 000*g* for 10 min to obtain the crude mitochondrial pellet. The final pellet was termed total mitochondria. Protein concentrations were determined using the BCA method.

Measurement of cytochrome C oxidase levels

Cytochrome C oxidase amounts were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Jianglai, Shanghai, China) according to the manufacturer's instructions. Briefly, 20 mg of tissue samples snap frozen in liquid nitrogen were maintained at 2–8°C after thawing. After the addition of PBS (pH 7.4), the samples were homogenized by grinders and subjected to centrifugation for 20 min at 3000 rpm. Supernatants were removed as soon as possible after specimen collection. Absorbance was read at 450 nm on a microplate reader. Cytochrome C oxidase concentration unit was showed as (pg/mg mitochondrial protein).

Determination of ATP levels by bioluminescence

Mitochondria from the brain regions of interest were isolated as described above. Then, ATP amounts were detected using an ATP determination kit (Beyotime) containing the firefly luciferase according to the manufacturer's instructions. Briefly, 20 mg of tissue was mixed with 100 µl test reagent, which catalyzes light production from ATP and luciferin. The emitted light was linearly related to ATP concentrations and measured on a microplate luminometer. ATP standard curves were generated in the range of 0.1–10 M. The relative luminescence activity was recorded for each sample and used to derive ATP concentrations on the basis of calibration curves.

Mitochondrial membrane potential assessment and imaging

Mitochondrial membrane potential was monitored quantitatively using the fluorescent dye tetramethylrhodamine ethyl ester (TMRE); excitation and emission wavelengths were 548 and 573 nm, respectively. Mitochondria (1 mg/ml) in PBS were incubated with TMRE (10 µM; Beyotime) for 15 min in the dark and washed three times with PBS. Fluorescence scanning of TMRE was monitored on a Thermo Scientific multiscan enzyme-labeled instrument (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

Alternatively, mitochondrial membrane potential was assessed using the lipophilic cationic probe JC-1 (Beyotime), a sensitive fluorescent dye. Briefly, a pure mitochondria solution was incubated with 10 µM JC-1 for 15 min at 37°C in the dark and monitored on an Olympus fluorescence microscope (Olympus Corp., Tokyo, Japan). Red fluorescence reflects the potential-dependent aggregation in the mitochondria. Green fluorescence, reflecting the monomeric form of JC-1, appears in the cytosol after mitochondrial membrane depolarization. Fluorescence measurements provided data in arbitrary units (AU).

Western blot analysis

Isolated mitochondria were lysed in extraction buffer containing protein-sparing modified fast (1 mM); mitochondrial fractions were boiled and separated by 12% SDS/PAGE (Bio-Rad, Beijing, China). The protein bands were electrophoretically transferred onto a nitrocellulose membrane (Servicebio, Wuhan, China). After blocking with TBST (20 mM Tris-HCl, 150 mM sodium chloride, 0.1% Tween-20) containing 10% (w/v) nonfat dry milk for 2 h at room temperature, the membrane was incubated overnight at 4°C with rabbit anti-AβPP antibody A8717 (Sigma-Aldrich, St Louis, Missouri, USA) at a 1 : 4000 dilution. This was followed by incubation with the secondary antibody (Abmart, Shanghai, China) at a 1 : 8000 dilution for 1 h at room temperature. Band intensities were analyzed using the ImageJ software (*http://imagej.nih.gov/ij/*).

ELISA for mitochondria-related Aβ and cortical total Aβ

Mitochondrial fractions (10–50 μg) and cortical tissue specimens (100 mg) were incubated in 5 M guanidine HCl and 50 mM Tris-HCl (pH 8.0) overnight; then, Aβ concentrations were detected with mouse Aβ1-42 ELISA kits KMB3441 (Invitrogen, Shanghai, China) following the manufacturer's instructions.

Electron microscopy

Frontal cortex specimens were immediately placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, sectioned to $\sim 1\,mm^2$, and incubated in the same glutaraldehyde solution for 12 h at room temperature. Samples were postfixed in 1% osmium tetroxide for 1.5 h, dehydrated in increasing concentrations of alcohol, immersed in propylene oxide, and embedded in Araldite 502 resin at 60°C. Ultrathin sections were placed on grids and stained with uranyl acetate and lead citrate. Thick sections were examined on a light microscope (Axioscop; Zeiss, Jena, Germany) and the grids with thin sections were evaluated under a transmission electron microscope (transmission electron microscopy, JEOL 1010; JEOL, Akishima, Japan).

Data analysis

Each experiment was conducted at least three times, and data are mean ± SE. Differences were evaluated using Student's *t*-test. A simple linear regression analysis was carried out to determine the relationships between ATP level and frontal cortical AβPP, frontal cortical Aβ, mitochondria-related AβPP, and mitochondria-related Aβ concentration; meanwhile, the associations of mitochondria-related Aβ levels with frontal cortical Aβ and mitochondria-related AβPP concentrations were assessed. *P* value less than 0.05 was considered statistically significant. All statistical analyses were carried out using the SPSS, 16.0 statistical software package (IBM Corp., Armonk, New York, USA).

Results

First, the patterns of mitochondrial morphology were determined by transmission electron microscopy. Compared with CO mice, CSR animals showed mitochondrial abnormalities, including a decrease in cristae density or even disappearance, vacuole formation by mitochondrial outer membrane extension, and intermembrane space expansion (Fig. 1).

Second, we analyzed mitochondrial function in the frontal cortex of CSR and CO mice by evaluating the cytochrome C oxidase concentration, ATP level, and mitochondrial membrane potential. Compared with CO mice, CSR mice showed a significantly lower cytochrome C oxidase concentration (8.722 ± 0.89 vs. 12.691 ± 1.58 pg/mg mitochondrial protein, $P < 0.05$; Fig. 2a) and lower ATP level (15.416 ± 6.90 vs. 52.965 ± 2.89 μM, $P < 0.05$; Fig. 2b). Meanwhile, CSR mice showed significantly lower mitochondrial potential than that of CO mice as the positive finding was confirmed by manifestations of mitochondrial potential dyed by TMRE and JC-1 (1.75 ± 0.71 vs. 2.64 ± 0.44 AU dyed by TMRE, 3.23 ± 0.38 vs. 3.90 ± 0.49 AU dyed by JC-1, respectively, both $P < 0.05$, Fig. 2c–e).

Third, ELISA was used to assess cortical mitochondria-related Aβ and AβPP levels, respectively. Mitochondria-related Aβ accumulation was significantly higher in the frontal cortex of CSR mice than that of CO mice (9.68 ± 0.93 vs. 5.30 ± 1.63 pg/mg, $P < 0.001$; Fig. 3a). CSR mice showed significantly higher levels of total Aβ (35.11 ± 5.55 vs. 25.27 ± 1.15 pg/mg, $P < 0.05$; Fig. 3b) than CO mice.

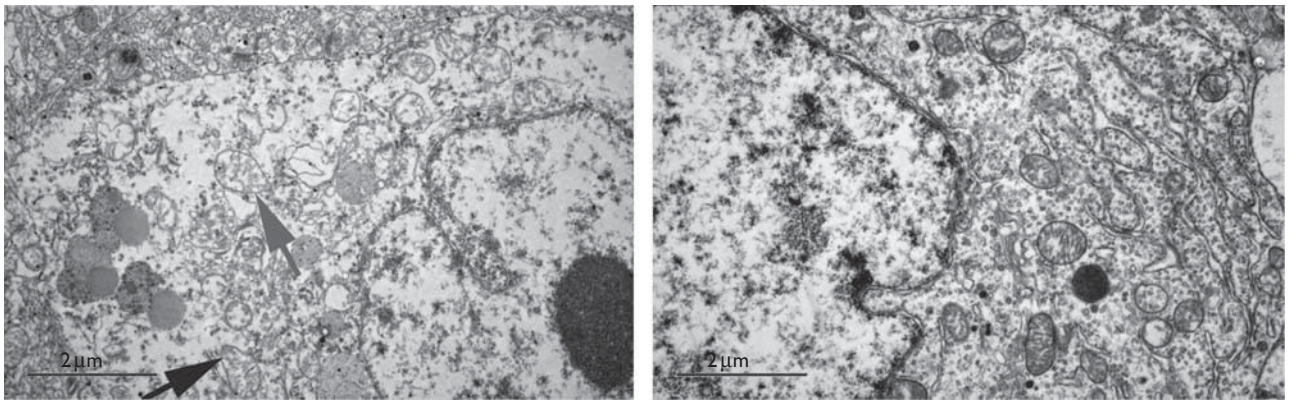
Fourth, Mitochondria-related AβPP levels were further assessed in both groups using the western blot method. Interestingly, CSR mice showed higher mitochondria-related AβPP levels compared with CO animals (1.18 ± 0.14 vs. 0.40 ± 0.07, $P < 0.001$) (Fig. 4).

In the chronic sleep-restriction model, mice frontal cortical mitochondrial dysfunction (reflected by cortical ATP level) correlated strongly with mitochondria-related Aβ and AβPP levels (determination coefficients: $r^2 = 0.787$, $P < 0.001$; $r^2 = 0.705$, $P < 0.001$, respectively; Fig. 5); however, the frontal cortical mitochondria-related Aβ level was not significantly related to cortical total Aβ ($P = 0.066 > 0.05$) or the mitochondrial AβPP concentration ($P = 0.084 > 0.05$).

Discussion

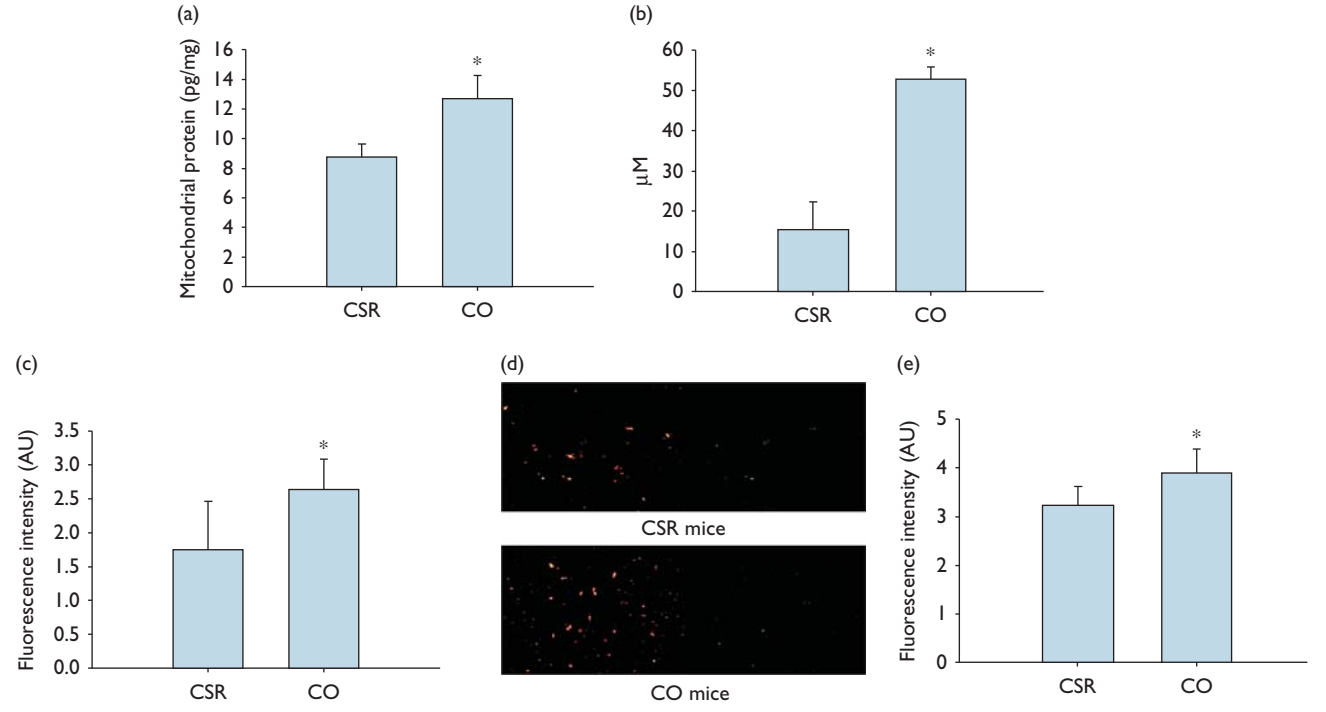
This study found that (a) CSR resulted in mitochondrial dysfunction in the frontal cortex of mice (as indicated by morphological changes in cellular organelles in electronic microscopic images), decreased mitochondrial respiratory chains complex concentration, and reduced ATP level and mitochondrial membrane potential; (b) under CSR, mitochondria-related Aβ and AβPP levels were significantly increased; (c) mitochondrial dysfunction

Fig. 1



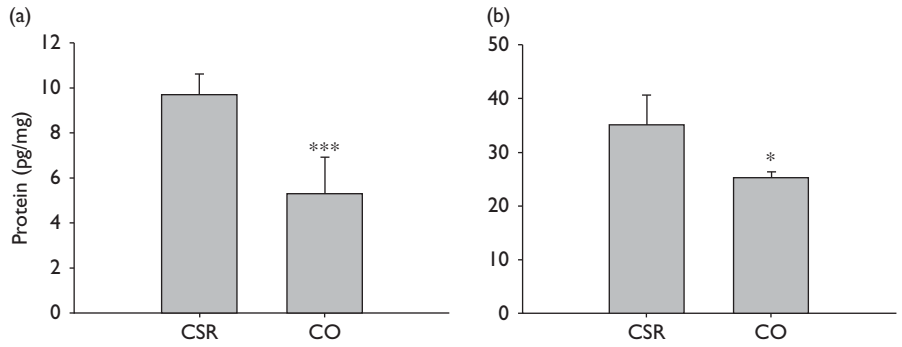
Frontal cortical neuronal abnormalities detected by transmission electron microscopy imaging. Compared with CO mice, the CSR animals showed mitochondrial abnormalities, including a decrease in cristae density or even disappearance, vacuole formation by mitochondrial outer membrane extension, and intermembrane space expansion (scale bar: 2 μm). CO, control; CSR, chronic sleep restriction.

Fig. 2



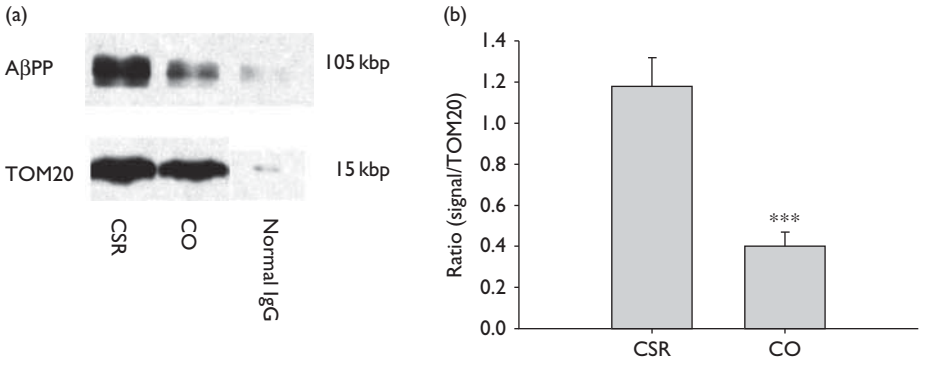
Mitochondrial function in the frontal cortex of CSR and CO mice. (a) Frontal cortical cytochrome C oxidase concentration. CSR mice showed significantly lower frontal cortical cytochrome C oxidase levels compared with CO mice (8.722 ± 0.89 vs. 12.691 ± 1.58 pg/mg mitochondrial protein; $^*P < 0.05$, $n = 5$). (b) Frontal cortical ATP level. CSR mice showed significantly lower frontal cortical ATP level in comparison with CO mice (15.416 ± 6.90 vs. 52.965 ± 2.89 μM; $P < 0.05$, $n = 5$). (c) Frontal cortical mitochondrial membrane potential. CSR mice showed significantly different mitochondrial membrane potential (1.75 ± 0.71 vs. 2.64 ± 0.44 AU) compared with CO mice after TMRE staining ($^*P < 0.05$, $n = 5$). (d) Frontal cortical mitochondrial membrane potential evaluated using the JC-1 method. (e) Frontal cortical mitochondrial membrane potential. CSR mice showed significantly lower mitochondrial membrane potential (3.23 ± 0.38 vs. 3.90 ± 0.49 AU) compared with the CO animals stained by JC-1 ($^*P < 0.05$, $n = 5$). CO, control; CSR, chronic sleep restriction; TMRE, tetramethylrhodamine ethyl ester.

Fig. 3



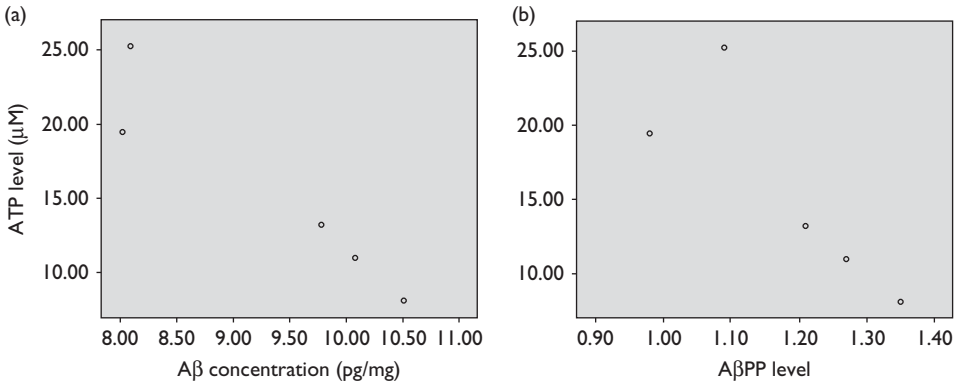
Frontal cortical mitochondria-related Aβ and total Aβ levels. (a) Frontal cortical mitochondria-related Aβ amounts. CSR mice showed significantly higher mitochondria-related Aβ levels (9.68 ± 0.93 vs. 5.30 ± 1.63 pg/mg) compared with CO mice (***$P < 0.001$, $n = 5$). (b) Frontal cortical total Aβ concentrations. CSR mice showed significantly higher total Aβ levels (35.11 ± 5.55 vs. 25.27 ± 1.15 pg/mg) compared with CO mice (*$P < 0.05$, $n = 5$). Aβ, β-amyloid; CO, control; CSR, chronic sleep restriction.

Fig. 4



Frontal cortical mitochondria-related AβPP levels. CSR mice showed significantly higher mitochondria-related AβPP levels in comparison with control animals (1.18 ± 0.14 vs. 0.40 ± 0.07; ***$P < 0.001$, $n = 5$). AβPP, β-amyloid protein precursor; CO, control; CSR, chronic sleep restriction.

Fig. 5



Correlation of ATP level and mitochondria-related Aβ/AβPP level. Mice frontal cortical mitochondrial dysfunction (reflected by cortical ATP level) correlated strongly with mitochondria-related Aβ and AβPP levels (determination coefficients: $r^2 = 0.787$, $P < 0.001$; $r^2 = 0.705$, $P < 0.001$, respectively). Aβ, β-amyloid; AβPP, β-amyloid protein precursor.

indicated by ATP level reduction was most correlated with mitochondria-related Aβ elevation; and (d) after a 2-month sleep restriction, mitochondria-related Aβ level was not significantly associated with mitochondria-related AβPP and total Aβ levels in the frontal cortex of 9-month-old C57BL/6J mice.

As an inevitable byproduct of modern society, CSR and its possible pathophysiological mechanism are rarely clarified, although individuals are paying increasing attention to sleep hygiene. Given the well-accepted findings of cognitive changes associated with sleep deprivation and the current prevalence of sleep disorders [11], investigation of the mechanism underlying the effect of sleep restriction on cellular physiology is of interest. In 1998, Cirelli *et al.* [18] found that after sleep deprivation for 3 h, the levels of RNAs encoded by the mitochondrial genome are uniformly higher during waking in many cortical regions. They hypothesized that the levels of mitochondrial RNAs might represent the regulatory response of the neural tissue in adapting to the increased metabolic demand of waking with respect to sleep. Recently, Nikonova *et al.* [19] assessed protein levels and enzyme activities of complexes IV and V of the electron transport chain in C57BL/6J mice after 3, 6, and 12 h of sleep deprivation. The increased complex IV protein amounts and enzyme activity after 3 and 12 h of sleep deprivation as well as the elevated complex V protein and enzyme activity after 12 h of sleep deprivation suggested dynamic energy regulation in the brain after acute sleep deprivation. However, just as Andreazza *et al.* [16] asked in their report: 'Does chronic sleep deprivation induce mitochondrial dysfunction?' or 'In which mitochondrial dysfunction is known to be involved?' The answers still remain unanswered in the CSR model. Our findings confirmed that CSR could cause mitochondrial dysfunction, which was reflected by reduced cytochrome C oxidase amount, ATP level, mitochondrial membrane potential, and mitochondrial morphological changes.

Using AβPP-transgenic mice, Kang *et al.* [10] confirmed that CSR (21 days) significantly increases Aβ plaque. In this study, 9-month-old wild-type mice subjected to a 60-day sleep restriction were selected for 2 reasons: (a) to show the effect of CSR on Aβ deposition and (b) to exclude the possible disturbance from abnormal AβPP expression to Aβ processing and mitochondrial function. As shown above, CSR led to a significant increase in the mitochondrial Aβ level. Given that a number of researches showed a key role for mitochondria-related Aβ in the process of AD [20–22], our findings not only showed that CSR gives rise to a series of mitochondrial dysfunctions but also that increasing levels of mitochondrial Aβ might be involved in the related pathological mechanism. To our knowledge, this is the first time that this finding has been reported. In addition, we found that CSR markedly increased mitochondria-related AβPP levels, with neuronal AβPP amounts remaining almost unchanged, which might be linked to the increasing burden of mitochondria-related Aβ.

Interestingly, different degrees of accumulation were found between mitochondria-related Aβ and total cortical Aβ levels in both groups. Compared with total Aβ levels, mitochondria-related Aβ amounts seemed to be more obviously increased in CRS mice than the total Aβ levels. Other findings [23] pointed out that mitochondrial Aβ was first detectable at 4 months of age, before significant extracellular deposition of Aβ in a transgenic mAβPP mice model. Reasonably, our findings can be explained as in the 6-month CRS mice model, Aβ peptides tended to accumulate in the mitochondria in the early stage of pathogenesis [24–26]. Mitochondria-related Aβ was found to be quite harmful. Dragicevic *et al.* [21] reported that Aβ oligomers extracted from mitochondria, rather than the whole cell, resulted in severe mitochondrial damage. The mitochondria-targeted pathophysiological mechanism of mitochondrial Aβ is involved in many aspects. Munguia *et al.* [27] reported that Aβ directly interacts with the intracellular protein endoplasmic reticulum amyloid β-peptide-binding protein, resulting in mitochondrial dysfunction and cell death. Other factors such as mitochondrial fragmentation, reduced neuronal mitochondrial density, and impaired complex III and IV activities have also been attributed to mitochondria-related Aβ [28]. On the basis of our results, frontal cortical mitochondrial Aβ elevation might be a reasonable cause of the cellular toxicity and mitochondrial dysfunction observed.

Our findings also showed that the association of mouse frontal cortical mitochondria-related Aβ concentration with the total Aβ concentration failed to reach significance under CSR. Similar results were found for mitochondria-related Aβ concentration and mitochondria-related AβPP levels. Excluding the small sample size ($n = 5$), these findings could be explained by the fact that the mitochondria-related Aβ concentration is not associated with either total Aβ levels or mitochondria-related AβPP amounts, corroborating a report by Mucke [29], who showed that Aβ accumulates in the mitochondria earlier than other intracellular and extracellular compartments. Actually, a more powerful confirmation is related to the way mitochondria-related AβPP is targeted to the mitochondrial membrane. Anandatheerthavarada and colleagues reported that AβPP is targeted to the mitochondrial outer membrane in an 'N-terminus-in mitochondria and C terminus-out cytoplasm' orientation [30]. In terms of the negative findings of AβPP in the isolated mitochondria [25], the notion that 'it is not likely to be produced locally in the mitochondria, and a complex cellular trafficking system is involved in importing Aβ into the mitochondria' is more reasonable. Further studies should explore the possible ways by which Aβ enters into the mitochondria.

Conclusion

Overall, CSR caused mitochondrial dysfunction in the frontal cortex of adult BL/6J mice, including reduced cytochrome C oxidase concentration, ATP level, mitochondrial membrane potential, and mitochondrial morphology changes in electron microscopy images. Moreover, CSR resulted in a significant mitochondria-related Aβ increase in the cortical region. These findings suggested that CSR-induced mitochondrial dysfunction might be related to frontal cortical mitochondria-related Aβ accumulation; the latter might be a pathophysiological change preceding Aβ deposition in any other compartments of the frontal cortex.

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Conflicts of interest

There are no conflicts of interest.

References

1 National Sleep Foundation. *2003 sleep in America poll*. Washington, DC: National Sleep Foundation; 2003.
2 Martins PJ, Nobrega JN, Tufik S, D'Almeida V. Sleep deprivation-induced gnawing-relationship to changes in feeding behavior in rats. *Physiol Behav* 2008; **93**:229–234.
3 Rechtschaffen A, Bergmann BM, Everson CA, Kushida CA, Gilliland MA. Sleep deprivation in the rat: I. Conceptual issues. *Sleep* 1989; **12**:1–4.
4 Trenell MI, Marshall NS, Rogers NL. Sleep and metabolic control: waking to a problem? *Clin Exp Pharmacol Physiol* 2007; **34**:1–9.
5 Campbell IG, Guinan MJ, Horowitz JM. Sleep deprivation impairs long-term potentiation in rat hippocampal slices. *J Neurophysiol* 2002; **88**:1073–1076.
6 Davis CJ, Harding JW, Wright JW. REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. *Brain Res* 2003; **973**:293–297.
7 Guan Z, Peng X, Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain Res* 2004; **1018**:38–47.
8 Kelleher RJ 3rd, Govindarajan A, Tonegawa S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* 2004; **44**:59–73.
9 Hipólide DC, Moreira KM, Barlow KB, Wilson AA, Nobrega JN, Tufik S. Distinct effects of sleep deprivation on binding to norepinephrine and serotonin transporters in rat brain. *Prog Neuropsychopharmacol Biol Psychiatry* 2005; **29**:297–303.
10 Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, *et al*. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science* 2009; **326**:1005–1007.
11 Huang Y, Potter R, Sigurdson W, Santacruz A, Shih S, Ju YE, *et al*. Effects of age and amyloid deposition on Aβ dynamics in the human central nervous system. *Arch Neurol* 2012; **69**:51–58.
12 Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001; **81**:741–766.
13 Cardinali DP, Pagano ES, Scacchi Bernasconi PA, Reynoso R, Scacchi P. Melatonin and mitochondrial dysfunction in the central nervous system. *Horm Behav* 2013; **63**:322–330.
14 Park HJ, Kim SS, Kang S, Rhim H. Intracellular Abeta and C99 aggregates induce mitochondria-dependent cell death in human neuroglioma H4 cells through recruitment of the 20S proteasome subunits. *Brain Res* 2009; **1273**:1–8.
15 Yang RH, Hu SJ, Wang Y, Zhang WB, Luo WJ, Chen JY. Paradoxical sleep deprivation impairs spatial learning and affects membrane excitability and mitochondrial protein in the hippocampus. *Brain Res* 2008; **1230**:224–232.
16 Andreazza AC, Andersen ML, Alvarenga TA, de-Oliveira MR, Armani F, Ruiz FS, *et al*. Impairment of the mitochondrial electron transport chain due to sleep deprivation in mice. *J Psychiatr Res* 2010; **44**:775–780.
17 Roman V, Hagewoud R, Luiten PG, Meerlo P. Differential effects of chronic partial sleep deprivation and stress on serotonin-1A and muscarinic acetylcholine receptor sensitivity. *J Sleep Res* 2006; **15**:386–394.
18 Cirelli C, Tononi G. Differences in gene expression between sleep and waking as revealed by mRNA differential display. *Brain Res Mol Brain Res* 1998; **56**:293–305.
19 Nikonova EV, Naidoo N, Zhang L, Romer M, Cater JR, Scharf MT, *et al*. Changes in components of energy regulation in mouse cortex with increases in wakefulness. *Sleep* 2010; **33**:889–900.
20 Caspersen C, Wang N, Yao J, Sosunov A, Chen X, Lustbader JW, *et al*. Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. *FASEB J* 2005; **19**:2040–2041.
21 Dragicevic N, Mamcarz M, Zhu Y, Buzzeo R, Tan J, Arendash GW, *et al*. Mitochondrial amyloid-beta levels are associated with the extent of mitochondrial dysfunction in different brain regions and the degree of cognitive impairment in Alzheimer's transgenic mice. *J Alzheimers Dis* 2010; **20** (Suppl 2):S535–S550.
22 Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum Mol Genet* 2006; **15**:1437–1449.
23 Chen X, Yan SD. Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer's disease. *IUBMB Life* 2006; **58**:686–694.
24 Dragicevic N, Smith A, Lin X, Yuan F, Copes N, Delic V, *et al*. Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. *J Alzheimers Dis* 2011; **26**:507–521.
25 Chen JX, Yan SS. Role of mitochondrial amyloid-beta in Alzheimer's disease. *J Alzheimers Dis* 2010; **20** (Suppl 2):S569–S578.
26 Fernández-Vizarra P, Fernández AP, Castro-Blanco S, Serrano J, Bentura ML, Martínez-Murillo R, *et al*. Intra- and extracellular Abeta and PHF in clinically evaluated cases of Alzheimer's disease. *Histol Histopathol* 2004; **19**:823–844.
27 Munguia ME, Govezensky T, Martinez R, Manoutcharian K, Gevorkian G. Identification of amyloid-beta 1-42 binding protein fragments by screening of a human brain cDNA library. *Neurosci Lett* 2006; **397**:79–82.
28 Tillement L, Lecanu L, Papadopoulos V. Alzheimer's disease: effects of β-amyloid on mitochondria. *Mitochondion* 2011; **11**:13–21.
29 Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, *et al*. High-level neuronal expression of abeta 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J Neurosci* 2000; **20**:4050–4058.
30 Anandatheerthavarada HK, Biswas G, Robin MA, Avadhani NG. Mitochondrial targeting and a novel transmembrane arrest of Alzheimer's amyloid precursor protein impairs mitochondrial function in neuronal cells. *J Cell Biol* 2003; **161**:41–54.