

Amyloid β and tau are involved in sleep disorder in Alzheimer's disease by orexin A and adenosine A(1) receptor

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Abstract. Sleep disorder is confirmed as a core component of Alzheimer's disease (AD), while the accumulation of amyloid β ($A\beta$) in brain tissue is an important pathological feature of AD. However, how $A\beta$ affects AD-associated sleep disorder is not yet well understood. In the present study, experiments on animal and cell models were performed to detect the association between sleep disorder and $A\beta$. It was observed that $A\beta$ 25-35 administration significantly decreased non-rapid eye movement sleep, while it increased wakefulness in mice. In addition, reverse transcription-quantitative polymerase chain reaction and western blot analysis revealed that the expression levels of tau, p-tau, orexin A and orexin neurons express adenosine A1 receptor (A1R) were markedly upregulated in the brain tissue of AD mice compared with that in samples obtained from control mice. Furthermore, the *in vitro* study revealed that the expression levels of tau, p-tau, orexin A and adenosine A1R were also significantly increased in human neuroblastoma SH-SY5Y cells treated with $A\beta$ 25-35 as compared with the control cells. In addition, the tau inhibitor TRx 0237 significantly reversed the promoting effects of $A\beta$ 25-35 on tau, p-tau, orexin A and adenosine A1R expression levels, and adenosine A1R or orexin A knockdown also inhibited tau and p-tau expression levels mediated by $A\beta$ 25-35 in AD. These results indicate that $A\beta$ and tau may be considered as novel biomarkers of sleep disorder in AD pathology, and that they function by regulating the expression levels of orexin A and adenosine A1R.

Introduction

As a neurodegenerative disease, Alzheimer's disease (AD) accounts for 50-70% of all dementia cases. With the aging of

the population, it is expected that the worldwide prevalence of AD will quadruple by 2050 as compared with the reported rate of 26.6 million cases in 2006, and ~43% of AD patients require a high level of care (1). The main symptoms of AD patients include cognitive decline, accompanied by psychological and behavioral abnormalities, decline in the ability to perform daily activities, depression and sleep disorder. As one of the common symptoms, sleep disorder severely affects the quality of life of patients (2).

AD-associated sleep disorder is increasingly prominent, and studies on mechanism of AD-associated sleep disorder and new therapies are of great value. However, the molecular mechanism of AD-associated sleep disorder remain unclear. Kondratova and Kondratov (3) have suggested that the sleep disorder in patients with AD may be associated with the degeneration of the suprachiasmatic nucleus, pineal gland, hypothalamus and brain nuclei. Furthermore, it has recently been reported that sleep disorder may accelerate the AD neurodegeneration and cognitive decline (4,5). A previous study has suggested that insufficient sleep facilitates the accumulation of amyloid β ($A\beta$), which has been confirmed as one of the main pathological characteristics of AD (6). Neurofibrillary tangles composed of an excessive amount of phosphorylated tau (p-tau) protein have been confirmed as the second pathology of AD. Therefore, $A\beta$ and abnormal tau protein have a central role in the neuropathology of AD (7,8). A previous study has also confirmed that a long tau was associated with the delayed sleep phase disorder, which may be the reflection of an abnormal circadian timing system (9). The interplay and synergy between $A\beta$ and tau induce sleep disorder and accelerate the pathogenesis of AD (10,11).

Sleep is produced when the internal inhibition process spreads to the cerebral cortex and subcortical structure. Sleep serves an essential role in the regulation of synaptic weight in the brain; for instance, it reduces the slow wave activity of the brain caused by the accumulation of synaptic enhancement in wakefulness (12). The neuropeptides orexin A (also known as hypocretin-1) and orexin neurons express A1 adenosine receptor (A1R) secreted from lateral hypothalamus (LH) neurons are known to be critical modulators of the sleep/wakefulness system (13,14). In addition, it has been reported that orexinergic system disorder may alter the sleep-wake rhythms and influence AD pathology (15). It has also been reported that blockade of adenosine A1R in the orexinergic

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LH induced a significant increase in wakefulness; however, adenosine A1R antagonists are able to reverse this effect and may serve as potential preventive or therapeutic strategies in AD patients (16,17).

Activation of adenosine A1R can accelerate sleep, whereas adenosine A1R blockade can promote awakening and compensatory sleep (17,18). A previous study has also reported that orexin A, which may increase arousal levels, promoted A β production and was associated with the regulation of the sleep-wake cycle. Furthermore, recent studies have focused on the associations of orexin A with p-tau (19). However, whether the changes in A β and p-tau levels are associated with orexin A secretion in the sleep-wake cycle remains unknown. Based on this preliminary evidence, the aim of the present study was to investigate the regulatory effect of A β and p-tau on sleep disorder in AD patients.

Materials and methods

Establishment of an AD animal model. Experiments were performed on male 5xFAD mice (15–25 g), which overexpressed human APP and PS1 with 5 familial AD mutations. 50 mice (1-year-old) were purchased from Kay Biological Technology (Shanghai) Co. Ltd (Shanghai, China). All the mice were free access to food and water and were kept in an undisturbed and clean environment with 12 h/12 h dark-light cycle, 90% relative humidity and 25°C. The animals were acclimatized to the laboratory environment for 1 week before the experiments. The AD mouse model was constructed as previously described in which 1-year old 5xFAD mice spontaneously exhibit AD-like pathological changes (20–22), and mice received cerebral injection of A β 25–35 (cat. no. ym-Y-0044, Yuanmu, China) 3 weeks prior to tests. Mice anesthetized with 400 mg/kg chloral hydrate (i.p.) (23) received an injection stereotactically, directly into the lateral ventricles, of aggregated A β 25–35 peptide (5 μ g/mouse i.c.v.) through a Hamilton syringe (VWR International, Fontenay-sous-Bois, France). Before use, A β 25–35 was diluted in sterile saline to a concentration of 0.5 mM and was maintained at 37°C for 7 days to pre-age the peptide (24). A water maze experiment was used to detect the behavioral changes of the mice. All animals were treated in accordance with the guidelines of Shandong University (Jinan, China), and experiments were approved by the Animal Research Committee of Shandong University.

Morris water maze. The Morris water maze test was conducted in a circular pool with a diameter of 120 cm, a height of 50 cm and a depth of 30 cm. The pool was divided into 4 quadrants, and a platform with a diameter of 10 cm and height of 28 cm was placed in the middle of one of the quadrants. The back of the head of the mouse was marked with picric acid for tracking. A marker that hung over the wall of the pool and a number of constant reference objects around the pool were used as visual cues. The water temperature was maintained at 25°C, and the hidden platform experiment was conducted 4 times per day for 5 successive days. Mice were dropped from 4 different locations and the escape latency time spent to find the hidden platform was monitored by a video tracking system. If the mouse did not find the hidden platform within 2 min, the researcher guided the mouse to the platform and permitted to

stay on the platform for 10 sec, after which the mouse was returned to the cage, and the incubation period was recorded as 2 min. The experiment consisted of two parts, in part 1, the mice were dropped into the 4 different quadrants, termed west (w), east (e), north (n) and south (s) respectively, each day for 5 days in the following order: Day 1, w-s-e-n; day 2, s-e-n-w; day 3: e-n-w-s; day 4, n-w-s-e; and day 5, w-s-e-n. In part 2, on day 6, the hidden platform was removed and the mice were put into the water from a quadrant that did not contain the platform. The number of times that the mice crossed the original location of the platform within 2 min was recorded. During part 1 and part 2, the total distance that the mice swam, and the time the mice spent in the platform quadrant were recorded.

Sleep analysis. The sleep disturbances and sleep-wake state of AD mice were recorded and analyzed with polysomnography, in which electroencephalogram (EEG) and electromyography (EMG) results were recorded and used to analyze the non-rapid eye-movement sleep and rapid eye-movement sleep. In order to obtain the EEG and EMG scan, EEG and EMG electrodes were implanted into the skull simultaneously (NeuroLogger, TSE Systems GmbH, Bad Homburg, Germany). For EEG recording, two stainless steel screws attached to wire electrodes were placed over the right frontal and parietal bones. For EMG recording, two wire electrodes were directly inserted into the neck musculature.

During the study, age matched mice were used as a control. Briefly, after 2 days of habituation to the device, 5xFAD mice and age matched controls underwent EEG recordings, which commenced on day 3. Body movements, and brain EEG activity was recorded and stored at 200 samples per second with a high-pass filter of 0.25 Hz and low-pass filter of 70 Hz. Digitized data were downloaded offline to a computer. EEG and EMG signals were recorded in a computer and then assessed with sleep-scoring software (SIRENIA® SEIZURE PRO).

Cell culture. The human SH-SY5Y cell line was purchased from the American Type Culture Collection (ATCC® CRL-2266™; Manassas, VA, USA) and cultured in Eagle's minimum essential medium (cat. no. M2279; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) supplemented with 10% fetal bovine serum (cat. no. F4135; Sigma-Aldrich; Merck KGaA), 100 IU/ml penicillin (cat. no. 10378016; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 mg/ml streptomycin (cat. no. 15140-122; Gibco; Thermo Fisher Scientific, Inc.), 1% non-essential amino acids (cat. no. 11140-050; Invitrogen; Thermo Fisher Scientific, Inc.) and 1% glutamine (cat. no. 11090-081; Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. SH-SY5Y cells were treated with or without the tau inhibitor TRx 0237. SH-SY5Y cells were grouped into negative control (SH-SY5Y) and A β 25–35 (SH-SY5Y+A β 25–35) or TRx 0237 (100 nM; cat. no. T7003; TargetMol, Boston, MA, USA) (SH-SY5Y+A β 25–35+TRx 0237). SH-SY5Y cells (1 \times 10⁵ cells/well) were seeded in 6-well plates and transfected with vehicle, A β 25–35 or TRx 0237 for 48 h. Before use, A β 25–35 was diluted in sterile saline to a concentration of 0.5 mM and was maintained at 37°C for 7 days to pre-age the peptide (24). The aged A β solution was diluted to 40 μ M for use.

Oligonucleotide transfection. Negative control (NC) and small interfering RNAs (siRNAs) targeting adenosine A1R (si-A1R) or orexin A (si-orexin A) were purchased from Shanghai GeneChem Co., Ltd. (Shanghai, China). SH-SY5Y cells (1×10^5 cells/well) were seeded in 6-well plates and transfected with NC, si-A1R or si-orexin A for 48 h using Lipofectamine 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Mice anesthetized with 400 mg/kg chloral hydrate (i.p.) (23) were decapitated on the day after all the behavioral and neurocognitive tests finished, and the whole brains of which olfactory bundle, optic nerve, cerebellum and medulla were removed were collected and frizzed at -80°C for PCR and western blotting assays. Total RNA from the brain tissues or cells was extracted using TRIzol reagent (cat. no. 15596-018; Invitrogen; Thermo Fisher Scientific, Inc.), cDNA was synthesized using $1 \mu\text{g}$ total RNA and then reverse transcribed to cDNA using an RT assay (DBI Bioscience, Newark, DE, USA). Subsequently, the relative expression of different target genes and controls was analyzed using a SYBR-Green PCR Master Mix kit (cat. no. RR420A; Takara Bio, Inc., Otsu, Japan), and the qPCR reactions were performed on an ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences for the genes were as follows: Tau, 5'-GTGGCCAGGTGGAAGTAAAA-3' (forward) and 5'-TGG AAGACACATTGCTGAGG-3' (reverse); orexin A, 5'-GCA TATCGGCCGCTTTAATA-3' (forward) and 5'-GGGTCC TCGAGTCTCTTTCC-3' (reverse); adenosine A1R, 5'-TGT AGGTGCCTTGGTCATCC-3' (forward) and 5'-ATCCCT GCTCTTCTTGCTGT-3' (reverse); GAPDH, 5'-GCCATC ACAGCAACACAGAA-3' (forward) and 5'-GCCATACCA GTAAGCTTGCC-3' (reverse). Cycling conditions included denaturation at 95°C for 2 min followed by annealing at 94°C for 20 sec for 40 cycles, and extension at 58°C for 20 sec. On the basis of exponential amplification of the target gene as well as a calibrator, the quantity of amplified molecules at the quantification cycle was given by $2^{-\Delta\Delta C_q}$. The data was assayed with the comparative $2^{-\Delta\Delta C_q}$ method (25) to determine the expression levels of target genes.

Western blot analysis. Total proteins from the tissues or cells were lysed in radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology, Haimen, China) containing protease inhibitors (BIOSS, Beijing, China), and the protein concentration was determined using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Equal amount of total proteins was then separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (cat. no. PK-NEF1002; PerkinElmer, Inc., Boston, MA, USA). Next, fat-free milk (5%) was used to block the membranes for 2 h at room temperature. Following blocking, the membranes were incubated with primary antibodies overnight at 4°C . The blots were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies (1:4,000) at room temperature for 1 h (Wuhan Boster Biological Technology, Ltd., Wuhan, China; cat. no. BA1054) and then developed using an enhanced chemiluminescence detection kit (Thermo

Fisher Scientific, Inc.) according to the manufacturer's protocol. The primary antibodies used included anti-GAPDH (dilution, 1:2,000; ab8245), anti-tau (dilution, 1:1,000; ab10439), anti-p-tau (dilution, 1:1,000; ab4841), anti-A1R (dilution, 1:1,500; ab82477) and anti-orexin (dilution 1:1,500; ab77370; all purchased from Abcam, Cambridge, MA, USA) antibodies.

Statistical analysis. The data were analyzed by the Student's t-test and analysis of variance (ANOVA) using IBM SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). The Student-Newman-Keuls post hoc test was used to calculate the P-value for pairwise comparisons following ANOVA. Paired t-test was used to analyze comparisons between the groups and paired data. Unpaired t-test was used to analyze comparisons between 2 groups. Each experiment was repeated at least 3 times. Data are presented as the means \pm standard deviation. A statistically significant difference was considered to be denoted by $P < 0.05$.

Results

Decreased non-rapid eye movement (NREM) sleep and increased wakefulness in AD mice. The sleep patterns in the control and AD groups were compared, and as mice are nocturnal animals, the sleep cycle was from 8 am to 8 pm. The results indicated that the NREM sleep in AD mice was significantly decreased compared with that in the control group ($P < 0.05$, $P < 0.01$ and $P < 0.001$; Fig. 1A). The results also demonstrated that non-rapid eye movement (NREM) sleep in AD mice was similar to the normal amount observed in control animals at other time points, except for at 16:00 ($P < 0.05$) (Fig. 1B). The wakefulness in AD mice at 8:00 was markedly increased compared with that of the control group ($P < 0.001$), however, it was equal to the normal amount of wakefulness observed in control animals at other hourly time points (Fig. 1C).

Behavioral changes in AD model mice. The Morris water maze test was applied to confirm the behavioral changes of AD mice. The tracing of the movement of mice during the hidden platform test and the probe trial test for the control and AD model mice are presented in Fig. 2. The results revealed that the escape latency of the hidden platform test was significantly longer in the AD model mice as compared with that in the control mice ($P < 0.05$; Fig. 2A and B). Therefore, it is suggested that the AD model was successfully established in the mice. In addition, the results indicated that the number of platform-crossings for the AD model mice was significantly lower compared with that of the control mice ($P < 0.05$; Fig. 2C and D). It is, thus, suggested that the learning ability of AD model mice was worse in comparison with that of control group mice.

Upregulation of tau, p-tau, orexin A and adenosine A1R in the brain tissue of 5xFAD mice. RT-qPCR and western blot analysis were conducted to validate the expression levels of different genes in the control and AD mice. The RT-qPCR results demonstrated that the relative expression levels of tau, orexin A and adenosine A1R were markedly upregulated in

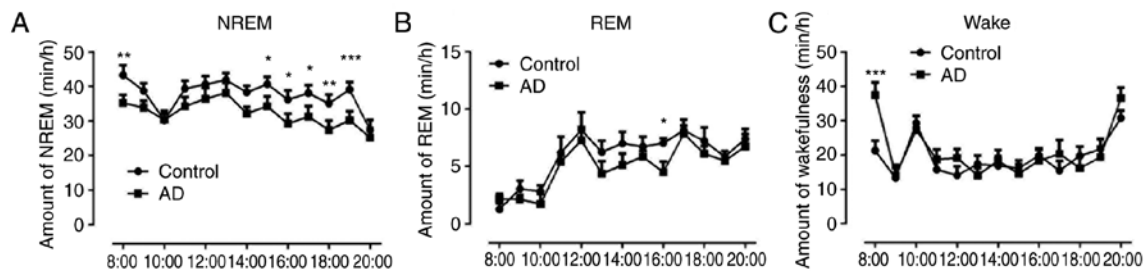


Figure 1. Changes of the sleep-wake cycle in 5xFAD mice. Hourly plots of (A) NREM sleep, (B) REM sleep and (C) wakefulness following treatment with or without A β 25-35 are shown. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. control group. NREM, non-rapid eye movement; REM, rapid eye movement.

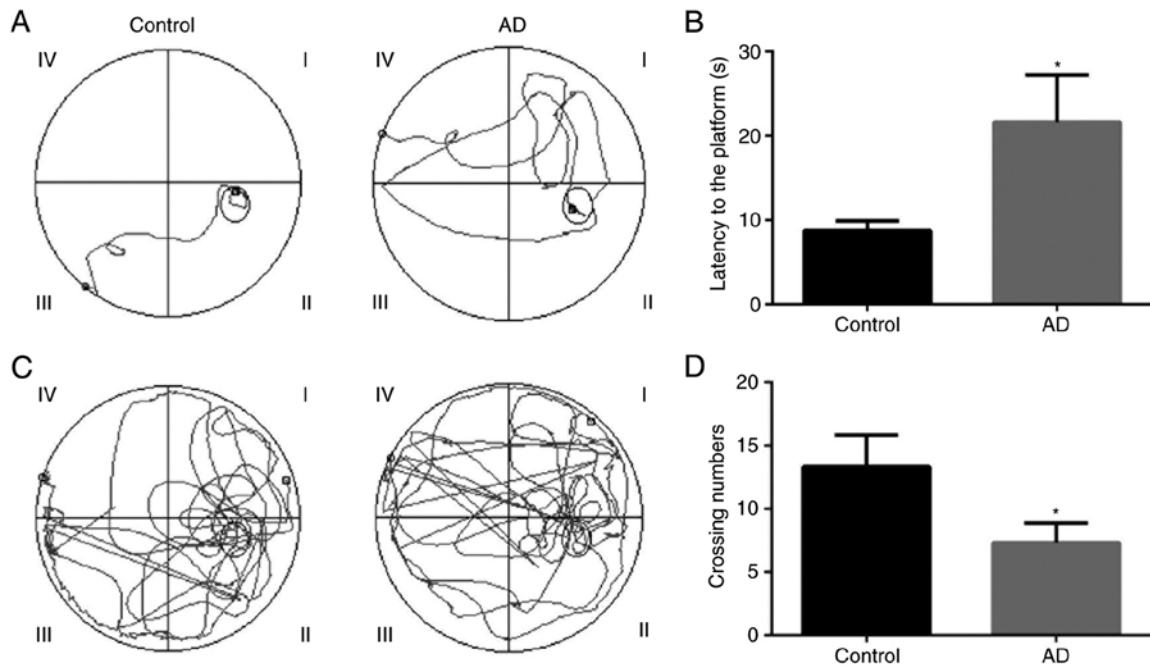


Figure 2. Deterioration of learning and memory in 5xFAD mice in the Morris water maze assay. (A) Tracked locations and (B) escape latencies of the control and AD model mice in the Morris water maze positioning navigation test. (C) Tracked locations and (D) platform-crossing number of the control and AD model mice in the probe trial tests. The Roman numerals I, II, III and IV represent the first, second, third and fourth quadrants, respectively, of the Morris water maze. Data are reported as the mean \pm standard error of the mean. * $P<0.05$ vs. control group. AD, Alzheimer's disease.

AD mice compared with the control mice ($P<0.01$, $P<0.001$ and $P<0.05$, respectively; Fig. 3A). The western blot results were consistent with the RT-qPCR results (Fig. 3B). The tau, p-tau, orexin A and adenosine A1R expression levels were higher in AD mice compared with those in control mice ($P<0.05$; Fig. 3C).

Treatment with A β 25-35 upregulates the expression levels of tau, p-tau, orexin A and adenosine A1R in SH-SY5Y cells. RT-qPCR and western blot analysis were also used to detect the mRNA and protein expression levels, respectively, in SH-SY5Y cells treated with A β 25-35. As shown in Fig. 4, the mRNA expression levels of tau, orexin A and adenosine A1R were significantly increased in A β 25-35-treated SH-SY5Y cells as compared with the control group cells ($P<0.01$), while significant increase was also observed in the protein levels of tau, p-tau, orexin A and adenosine A1R.

Changes in mRNA and protein expression levels following treatment with A β 25-35, or a combination of A β 25-35 and

TRx 0237. There was a significant increase in the mRNA relative expression levels of adenosine A1R and orexin A following A β 25-35 treatment; however, the high expression levels of adenosine A1R and orexin A were significantly reduced following A β 25-35 and TRx 0237 co-treatment ($P<0.05$; Fig. 5A). Next, the protein expression levels of tau, p-tau, orexin A and adenosine A1R were determined by western blot analysis. Overexpression of tau, p-tau, orexin A and adenosine A1R was observed in the A β 25-35-treated group compared with the control group. However, TRx 0237 co-treatment significantly reversed the promoting effects of A β 25-35 on tau, p-tau, orexin A and adenosine A1R expression (Fig. 5B and C).

Silencing of adenosine A1R and orexin by siRNA transfection inhibits tau and p-tau expression mediated by A β 25-35 in SH-SY5Y cells. The current study further explored the effects of adenosine A1R and orexin A knockdown on tau and p-tau expression induced by A β 25-35 in the AD cell model. As shown in Fig. 6, the results indicated that tau and p-tau

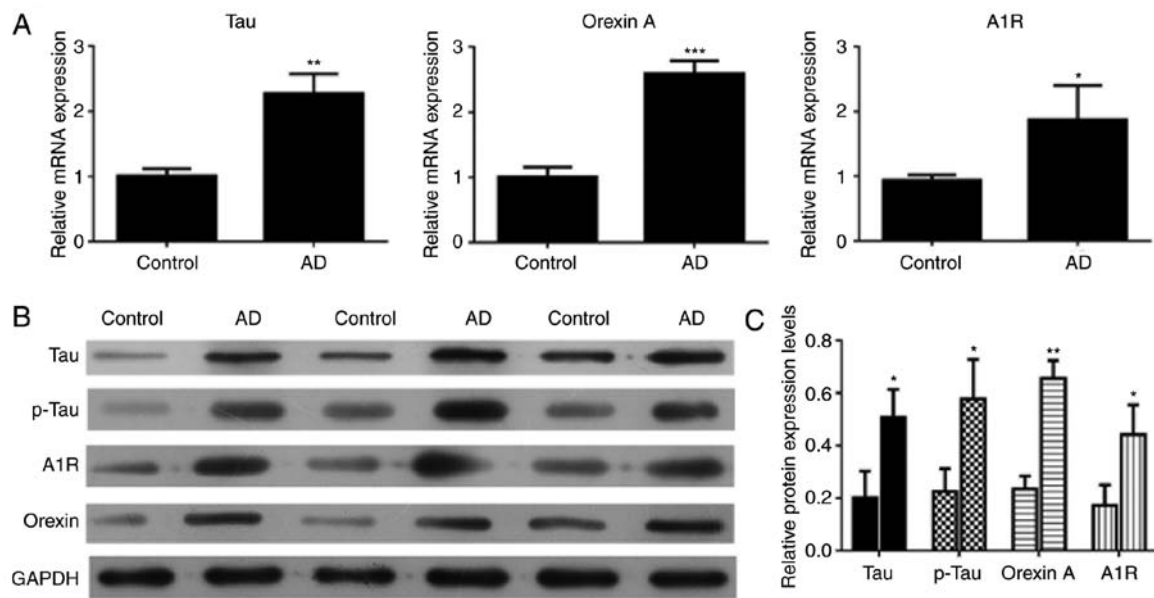


Figure 3. Effect of A β 25-35 administration on the expression levels of tau, p-tau, orexin A and A1R in the brain tissue of mice. (A) Reverse transcription-quantitative polymerase chain reaction analysis of tau, orexin A and A1R mRNA levels in the control and AD mice. (B) Western blots and (C) quantified gray values of tau, p-tau, orexin A and A1R protein expression levels in the control and AD mice. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control group. AD, Alzheimer's disease; p-tau, phosphorylated tau.

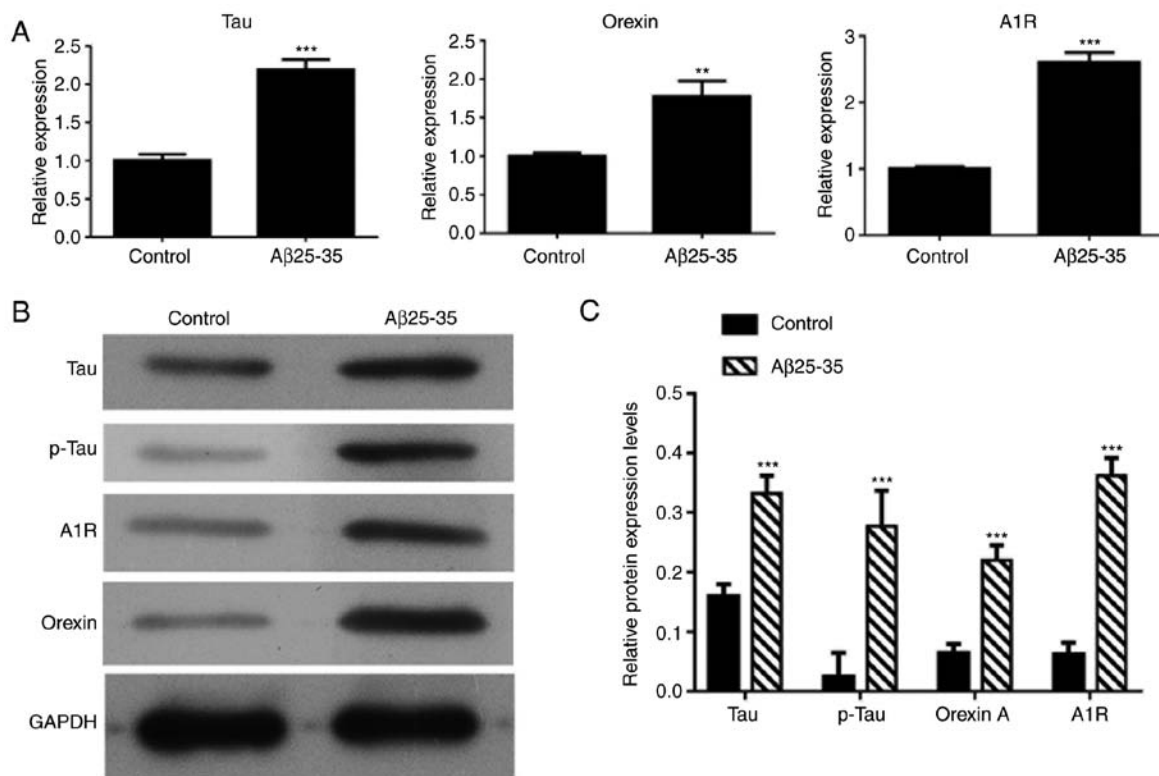


Figure 4. Effect of A β 25-35 treatment on the expression levels of tau, p-tau, orexin A and A1R in SH-SY5Y cells. (A) Relative tau, orexin A and A1R mRNA expression levels in cells with or without A β 25-35 treatment. (B) Western blots and (C) grayscale values of tau, p-tau, orexin A and A1R protein expression levels in cells with or without A β 25-35 treatment. Each experiment was repeated at least 3 times. ** $P < 0.01$ and *** $P < 0.001$ vs. untreated cell group. p-tau, phosphorylated tau.

expression levels were significantly upregulated in the A β 25-35 group compared with the control. However, these levels were significantly downregulated in the A β 25-35 + si-A1R and A β 25-35 + si-orexin A groups compared with the A β 25-35 and

A β 25-35 + NC groups ($P < 0.05$; Fig. 6). Therefore, these data suggested that adenosine A1R or orexin knockdown inhibited the A β 25-35-mediated expression levels of tau and p-tau in the AD cell model.

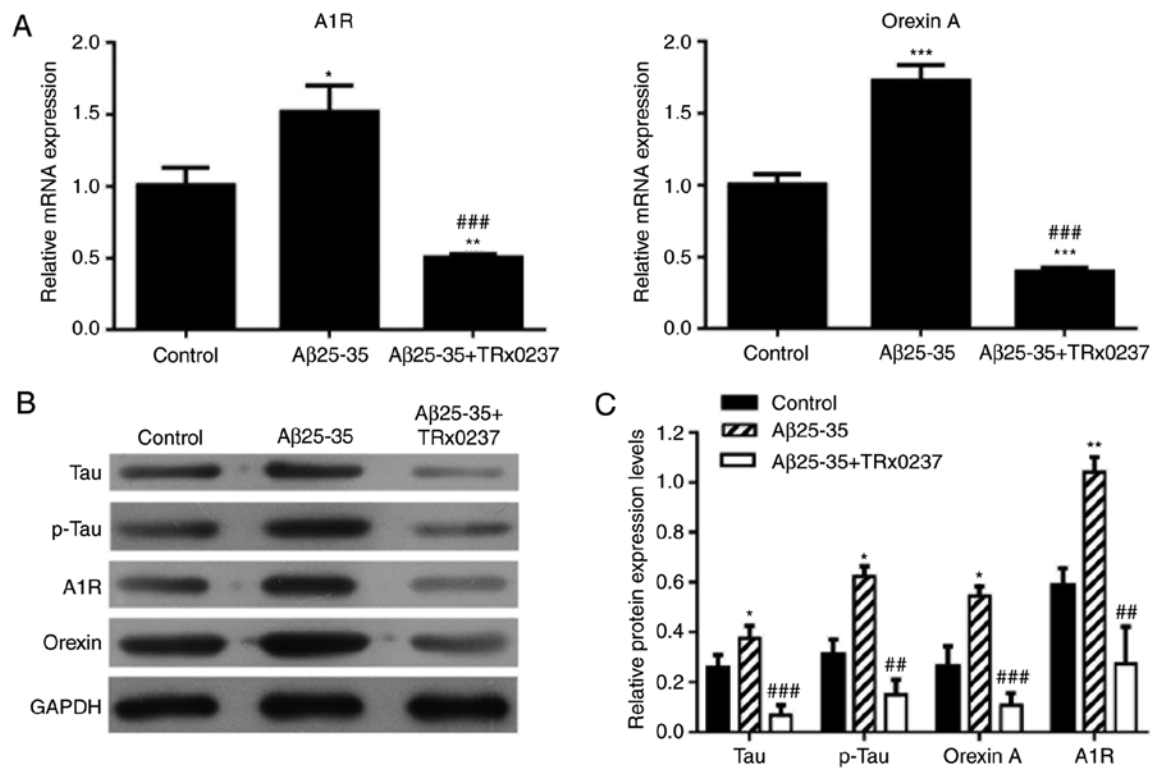


Figure 5. Changes in the mRNA and protein expression levels in SH-SY5Y cells following treatment with A β 25-35 or with a combination of A β 25-35 and TRx 0237. (A) Gene expression changes for A1R and orexin A upon treatment with A β 25-35 or A β 25-35+TRx 0237. (B) Western blots and (C) quantified values of the protein expression levels of tau, p-tau, orexin A and A1R following treatment with A β 25-35 or A β 25-35+TRx 0237. Each experiment was repeated at least 3 times. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control group; ## $P < 0.01$, ### $P < 0.001$ vs. A β 25-35-treated group. p-tau, phosphorylated tau.

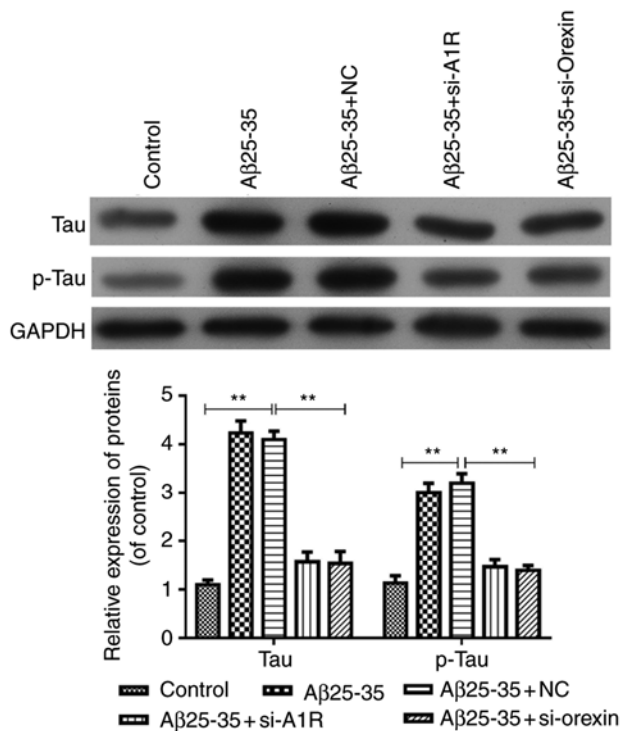


Figure 6. Silencing of A1R and orexin A by siRNA transfection inhibited tau and p-tau expression mediated by A β 25-35 in the Alzheimer's disease cell model. SH-SY5Y cells were treated with A β 25-35 and then transfected with NC, si-A1R or si-orexin A, respectively. Protein expression levels of tau and p-tau were measured by western blot assay and quantified according to the protein gray values in treated SH-SY5Y cells. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control group; ### $P < 0.001$ vs. A β 25-35+NC group. p-tau, phosphorylated tau; siRNA, small interfering RNA.

Discussion

AD is the most common type of neurodegenerative disease that affects the cognitive functions of the elderly (26). Currently, no therapies are available to halt or reverse the progression of this disease. In the present study, it was observed that NREM sleep was decreased and wakefulness was increased in AD mice. In the Morris water maze assay, a shorter escape latency time indicates better memory, and increased number of platform crossings indicates better learning ability (27,28). The present study in 5xFAD mice revealed a longer escape latency and lower number of platform crossings compared with control group, which indicated a deteriorated memory and learning ability in 5xFAD mice.

A β serves a pivotal and potentially causative role in the pathogenesis of AD, and has thus become a major therapeutic target (29). At present, studies have confirmed that AD neurodegeneration started with the aggregation of non-soluble monomeric A β peptides, and that the deposition of A β in plaques is the main driver of AD pathogenesis. Epidemiological studies have reported that patients with AD suffered from sleep dysfunction (2,30,31). Sleep disorder has been confirmed to decrease clearance of A β in the brain and accelerate AD pathology. In the present study, the interaction between sleep disorder and AD was further explored.

In recent years, it has been documented that the aggregation of A β can induce abnormal phosphorylation of tau protein and lead to nerve fiber formation (32). In addition, a higher level of tau protein has been confirmed as a marker of rapid cognitive decline, while the abundance of neurofibrillary

tangles was correlated with the severity of dementia (33,34). Previous studies (20-22) investigated neurocognitive function of the 5xFAD mice, using an AD animal model with a Morris water maze assay. In addition, in a study performed by Kang *et al* (10) suggested that the levels of soluble A β are positively associated with the time awake and negatively associated with the time asleep. In the present study, the 5xFAD mice AD model was used, and the data indicated a significantly decreased NREM sleep and increased wakefulness in the mice.

The orexinergic system is a key regulator of sleep onset. The neuropeptide orexin A (also known as hypocretin-1) produced by LH neurons has a significant impact on sleep-wake cycle regulation (13,35). It has been suggested that orexin regulated both the diurnal wake and nocturnal sleep periods through reducing REM sleep and slow-wave sleep, and increasing wakefulness (36,37). Additionally, adenosine inhibits excitatory synaptic transmission of orexin neurons via the receptor adenosine A1R. Blockade of adenosine A1R in the orexinergic LH induced a significant increase in wakefulness, while it decreased sleep in the sleep-wake cycle (16,17). Consequently, it can be predicted that the sleep disorder may be associated with the abnormal regulation of A β and tau on the adenosine A1R axis. In the present study, the expression levels of orexin A and adenosine A1R were found to be markedly upregulated in the brain tissue of AD mice, and similar results were observed in SH-SY5Y cells treated with A β 25-35. In addition, treatment with the tau inhibitor TRx 0237 not only decreased the tau and p-tau expression levels, but also reversed the promoting effects of A β 25-35 on orexin A and adenosine A1R expression levels. Adenosine A1R or orexin knockdown also inhibited tau and p-tau expression mediated by A β 25-35 in the AD cell model. In fact, a previous publication indicated that increased orexin A promoted wakefulness and sleep fragmentation, consequently promoting p-tau accumulation (38). In the current study, it was confirmed that orexin A expression was positively correlated with the wakefulness time and tau expression induced by A β accumulation, which provides further evidence on the regulatory network of sleep disorder in AD pathology.

In conclusion, the present study highlighted that sleep disorder in 5xFAD mice with AD-like neurocognitive changes is positively associated with the accumulation of A β and tau, which induced the overactivation of the orexinergic system. Therefore, these findings suggest that A β and tau can be considered as novel biomarkers of sleep disorder in AD pathology. Furthermore, A β and tau appear to serve key roles in sleep regulation by regulating the expression levels of orexin A and adenosine A1R. However, further studies are required to validate how silencing A1R and orexin by siRNA transfection affects the sleep-wake cycle changes. It is also necessary to investigate the deeper mechanisms underlying the actions of adenosine A1R and orexin in sleep disorder in AD.

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Availability of data and materials

The data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZL, FW and MT conceived and designed the experiment. ZL, FW, MT, YZ and XW carried out the experiments. ZL, FW, and MT acquired the reagents and materials. ZL and FW analyzed the data. ZL FW and MT wrote the manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate

All animals were treated in accordance with the guidelines of Shandong University (Jinan, China), and experiments were approved by the Animal Research Committee of Shandong University.

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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