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Original Article

Memory consolidation and inducible nitric oxide synthase expression during different sleep stages in Parkinson disease

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ABSTRACT

Background: Parkinson disease (PD) is a neurodegenerative disease characterized by motor and nonmotor dysfunctions, which include sleep disturbances. Rapid eye movement (REM) sleep is associated with numerous physiologic changes such as memory consolidation. Compelling evidence suggests that nitric oxide (NO) is crucial to both sleep regulation and memory consolidation. In our study, we explored changes in biologic molecules during various sleep stages and the effects of sleep on memory consolidation in PD.

Methods: Ten PD patients and 14 volunteers without PD participated in our study. The gene expression of inducible NO synthase (iNOS) in all sleep stages was measured using realtime polymerase chain reaction (PCR) based on polysomnography (PSG)-guided peripheral blood sampling. In addition, the efficiency of memory consolidation during the sleep of the participants was measured using the Wechsler Memory Scale, third edition (WMS-III).

Results: The iNOS expression increased in all sleep stages among the PD patients compared to the control participants, in whom iNOS expression decreased during REM sleep. Regarding memory consolidation, the performance of the controls in logic memory and the patients in visual reproduction tasks improved after sleep.

Conclusions: The iNOS synthase expression was different from control participants among PD patients, and the expression was dissimilar in various sleep stages. Sleep might enhance memory consolidation and there are different memory consolidation profiles between PD and control participants demonstrating distinct memory consolidation profiles.

1. Introduction

Parkinson disease (PD) is a neurodegenerative disease characterized by an insidious onset and slow progression of rigidity, tremors, bradykinesia, and balance impairment. In addition to motor dysfunction, PD patients may exhibit nonmotor symptoms, such as sensory symptoms, autonomic dysfunction, and sleep disturbances. Several sleep disturbances are particularly common in PD patients, including insomnia, nightmares, sleep apnea, rapid eye movement (REM) sleep behavior disorder, restless legs syndrome, periodic limb movement disorder, and excessive daytime sleepiness [1]. All of these symptoms can occur prior to the onset of PD motor symptoms and typically deteriorate during the disease process. The neurotransmitter dopamine plays a role in producing a wakefulness-promoting effect in vertebrates. Dopaminergic deficiency might be attributed to these sleep disturbances. Other systems, such as the serotoninergic, cholinergic, and norepinephrinergic systems, also play roles in sleep, but few studies have focused on PD sleep disorders [2]. All of these neurotransmitters, which play key roles in neurodegenerative diseases, are involved in the initiation and maintenance of sleep.

Sleep affects behaviors by altering endocrine, neurotransmitters, and intracellular molecular events [3]. REM sleep, in which PD patients commonly exhibit abnormalities, accompanies numerous physiologic changes, such as a decrease in muscle tone, an increase in brain activities, and irregular cardiopulmonary functioning. These REM-related changes also are associated with

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dramatic changes in the microenvironment of the brain, including gene-expression patterns and activities of neurotransmitters [4]. In addition, sleep is essential for maintaining neurocognitive functions including memory consolidation [4–6]. Distinct memory is linked to unique sleep-related consolidation mechanisms that work during various sleep stages in REM sleep or slow-wave sleep (SWS) and in different brain regions throughout the night [7].

Increasing evidence suggests that nitric oxide (NO), originally identified as endothelium-derived relaxing factor also is crucial for both sleep regulation and memory consolidation [7]. Four forms of NO synthase are responsive to the generation of NO, which are neuronal (nNOS), endothelial, mitochondrial, and inducible (iNOS). Three processes regulate NO synthesis and NOSs activities, specifically, the L-arginine/arginase substrate-competing system; the citrulline/L-arginine-recycling system; and the asymmetric dimethylarginine/dimethylarginine dimethylaminohydrolase NOSs-inhibiting system. Researchers have documented that nitrites and nitrates may back-generate NO under ischemic conditions. This process must be considered regarding patients with neurodegenerative diseases such as PD [8]. A previous study reported that the presence of NO in the brain facilitates sleep, particularly REM sleep, whereas the presence of NO in the periphery may inhibit sleep [8]. In an nNOS/iNOS knockout (KO) young animal study, nNOS and iNOS demonstrated opposite effects on sleep; nNOS KO mice experienced a decrease in REM sleep, and iNOS KO mice exhibited an increase in REM sleep [9]. In contrast to the young animal study, another study indicated that iNOS may be essential to both the initiation and maintenance of REM in aging animals [10]. These studies have suggested that the role of the NO system in sleep regulation is complex. L-NG-nitroarginine methyl ester, an NOS inhibitor, was revealed to induce impairments in immediate, short-, and long-term memories of inhibitory avoidance tasks in animals. The iNOS expression was discovered to substantially increase in brains affected by PD, and this change in the NO system might affect sleep architecture and contribute to the clinically observed sleep and behavioral disturbances in PD patients.

We designed our study to explore changes in biologic molecule, iNOS, during various sleep stages in PD patients. In addition, we measured the efficiency of memory consolidation during sleep in PD patients. The central molecules were indirectly examined by sampling peripheral leukocytes, based on the concept of some data supporting the synchronized expression of circadian genes between the central circadian clock and peripheral organs, including the leukocytes [11,12]. Thus we investigated the dissimilarities in iNOS gene expression between PD patients and non-PD control participants in REM sleep, SWS sleep, and wake stages by using polysomnography (PSG)-guided peripheral blood sampling.

2. Methods

2.1. Participants

Ten PD patients and 14 non-PD volunteers aged 46–68 years were recruited from Taipei Medical University Hospital (TMUH) and Taipei Medical University Shuang-Ho Hospital. The PD diagnosis was based on the UK Parkinson's Disease Society Brain Bank criteria. The severity of PD in patients was evaluated using the Hoehn and Yahr Scale. Among the participants, nine patients were in Stage 1 of PD and one patient was in Stage 2. All of the participants provided written informed consent, which was approved by the Joint-Institutional Review Board of Taipei Medical University. Participants who possessed a history of sleep disturbance or were regularly taking hypnotic drugs were excluded from our study.

2.2. Study design and memory tests

All of the participants arrived at the sleep center of TMUH or Taipei Medical University Shuang-Ho Hospital at approximately 9:00 pm and were asked to provide basic information. They performed the first recall tasks by using the Wechsler Memory Scale, third edition (WMS-III) until 10:00 pm and then underwent PSG after an intravascular catheter was inserted into the cubital vein for further blood sampling. The participants fell asleep in individual rooms at approximately 11:00 pm. They were awoken by light exposure at approximately 6:00 am. Shortly afterward they performed the second recall tasks, which were identical to those performed the previous night (Fig. 1).

To evaluate the relationship between sleep architecture and gene expression with memory consolidation in PD patients, the episodic memory tasks, which were designed and defined according to the WMS-III, were included in our study. These tasks comprise a logical memory task, a verbal-paired association task, and a visual reproduction task. All of the tests were conducted by a single member of the medical personnel according to manufacturer instructions.

After the participants learned all four of the tasks included in the WMS-III, they were required to perform the first recall prior to sleeping. After approximately 8 h of sleep, they were asked to recall all of the tasks, which were learned the previous day, without being provided any information regarding these tasks; this process was defined as the second recall tasks. The results of recall performance and those of memory consolidation are represented by the percentage of memory retention.

2.3. PSG and blood sampling

A PSG examination was conducted on all of the participants by using a Sandman Elite (Tyco Healthcare, Canada) at the sleep center of TMUH, or by using an Embla N7000 (ResMed, Australia) at Shuang-Ho Hospital. Prior to the scheduled sleep at 11:00 pm, venous catheters were inserted into the forearm veins of the participants. During the PSG examination, three samples that each contained 3 cc of peripheral venous blood were collected at three end points: (1) the first REM sleep, (2) non-REM SWS following the REM sleep, and (3) 15 min after the end of the PSG examination when the participants were awoken at 7:00 am by light exposure. The scoring of the PSG examination was performed by qualified PSG technicians based on the Rechtschaffen and Kales rules. Table 1 lists the PSG parameters.

2.4. RNA extraction and realtime polymerase chain reaction

After collecting peripheral blood, the blood was immediately transferred to RNAlater® RNA Stabilization Reagent kits (Qiagen; Valencia, CA, USA) to prevent RNA degradation. The total RNA was harvested using an RNA Extraction RiboPure-Blood kit (Ambi-

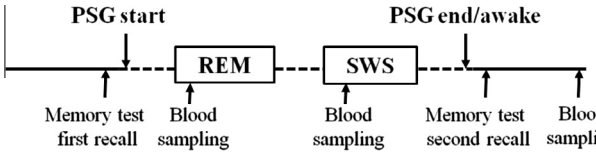

Fig. 1. Study design. Participants underwent memory tests before and after polysomnography (PSG), and blood samples were collected at three end points based on the PSG findings.