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分担研究報告書

睡眠物質のヒト睡眠・覚醒リズムに与える影響に関する研究

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研究要旨

睡眠物質の一つであるメラトニンは、概日リズム睡眠障害に有効であるとする報告がなされているが、作用機序については結論が出ていない。そこで、メラトニンのヒト睡眠・覚醒リズムに与える影響について、masking effect の極力少ない方法を用いて、脳波解析を行った。その結果、急性効果としての催眠効果と、２日目以降の生物リズムを前進させる作用があることが明らかになった。ただしこれらの作用は、メラトニンを投与する時刻依存性があり、臨床応用するには、投与のタイミングが重要であると考えられた。

A.　研究目的

概日リズム睡眠障害では、生物リズムが 24 時間のリズムに同調できなくなり、その結果睡眠・覚醒リズムがずれてしまう。この症候群対する時間生物学的治療法として、高照度光療法、メラトニン投与などがある。高照度光療法は生物リズムの位相を変化させることによって、24 時間の昼夜リズムに同調させる事ができる。睡眠物質の一つであるメラトニン投与も概日リズム睡眠障害に有効であると報告されている。その作用機序としては、生体リズムの位相を変化させる作用や睡眠促進作用などが推測されているが、一定の結論は出ていない。そこで、メラトニンの概日リズム睡眠障害に対する作用機序を明らかにすることを目的に、メラトニンを投与して生物リズムの指標と脳波解析を同時に行った。

B.　研究方法

対象は健康成人男性８名（20～25 歳、平均年齢 22.8 歳）である。実験に先立ち、１週間前から規則正し

い生活をするように指示し、アクチウォッチ（Mini Mitter 社）を用いて連続活動量記録を行い、これを確かめた。実験1日目は午前 11 時に集合し昼食をとらせた後、200lux の実験室内で安静を保たせた。実験1日目の 16 時から実験 4 日目の 21 時までの 77 時間の間、20 分-40 分の超短時間睡眠覚醒スケジュール法を1週間の間隔をあけて2セッション施行した。超短時間型睡眠覚醒スケジュール法は、60 分を1サイクルとし、40 分間は実験室において座位安静を保たせ、20 分間はシールドルーム内で安静臥床させ（nap trial）脳波記録（C3-A2, C4-A1, O1-A2）を行うという方法である。実験2日目の 19 時と 21 時に各々偽薬またはメラトニン 0.5mg をシングルブラインドにて経口投与した。Nap trial における睡眠段階 2,3,4 およびレムが出現している時間帯の脳波 C3-A1 誘導において、高速フーリエ変換によるパワースペクトラム解析をおこなった（low-pass filter は 0.5Hz、high-pass filter は 200 Hz）。同時に、1時間毎に唾液メラトニン濃度を測定した。実験中は直腸温を連続して2分ごとに測定した。実験室内は温度 24℃、湿度 60%、照度 10lux 以下に、脳波検査中のシールドルーム内は照度 1lux 以下に保った。実験中は 2 時間ごとに 150kcal の栄養食品と 200cc のカフェインを含まないノンカロリーの飲み物を与えた。

本研究は国立精神・神経センター国府台地区の倫理委員会の承認を得ており、対象車内は本研究に関して十分な説明を行い、文書にて同意を取得した。

C. 研究成果

偽薬投与条件下で得られた3周期分の delta power, theta power を分散分析で検討すると、周期（日）による効果はみられず、1日の時刻による効果のみ認められ、日と時刻の交互作用もみられなかった。これは本実験において、睡眠中の delta power, theta power 自体が概日リズムを持つことを示すという結果であった。メラトニン投与条件と偽薬投与条件を比較検討すると、外因性メラトニンを投与した時間帯に一致して、メラトニン投与条件では delta power、theta power とも有意に低下していた。個々のケースを検討すると、外因性メラトニンによる唾液中メラトニンが高濃度の時刻が、生理的なメラトニン分泌時刻からずれている例では、その時刻に一致して delta power, theta power のいずれも高値を示していたのに対して、外因性メラトニンによる唾液中メラトニンが高濃度の時刻が、生理的メラトニン分泌時刻と一致している例では、delta power, theta power とも変化は認められなかった。また、投与2日目には、メラトニン投与条件では delta power、theta power とも早く出現・減衰するパターンがみられた。

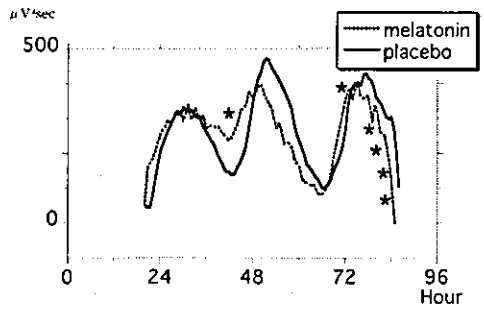

図1　delta power

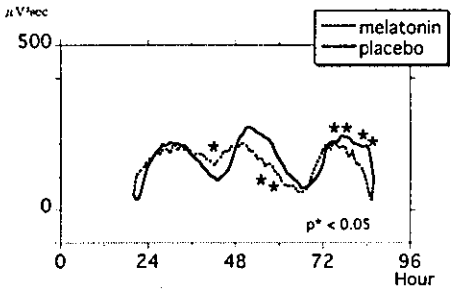

図2　theta power

D.　考察と結論

今回の結果からは、外因性メラトニン投与の急性効果として、delta power、theta power ともに増加させており、催眠作用があるとともに睡眠内容も良好にさせる作用があることが示唆された。われわれの行った先行研究から、メラトニン濃度と深部体温、末梢からの放熱の指標である DPG（Distal-Proximal Gradient）が相関関係にあることが示されている。こうしたことから、末梢からの放熱を増加させ深部体温を低下させる作用が、メラトニンの睡眠に与える作用機序であることが、メラトニン投与実験からも明らかになった。ただし、内因性メラトニンが分泌されている時間帯ではこのような結果が得られなかったことから、睡眠物質であるメラトニン投与の急性効果は、投与するタイミングが重要であると考えられた。また、メラトニン投与は、2日目の delta power、theta power のリズムを前進させており、睡眠・覚醒リズムを前進させる作用も併せ持つことが示された。今後、概日リズム睡眠障害にメラトニン投与を応用していく場合には、この二つの作用機序に応じて、投与するタイミングを決定していく必要があると考えられた。

E.　健康危機情報

なし

F. 研究発表

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E.　非的財産権の出願・登録状況

なし

F.　特許取得

なし

III. 研究成果の刊行に関する一覧表

刊行物一覧

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主要な刊行文献

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Dreaming During Non-rapid Eye Movement Sleep in the Absence of Prior Rapid Eye Movement Sleep

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Study Objectives: There is a long-standing controversy surrounding the existence of dream experiences during non-rapid eye movement (NREM) sleep. Previous studies have not answered the question whether this "NREM dream" originates from the NREM sleep mechanism because the subject might simply be recalling experiences from the preceding rapid eye movement (REM) sleep.

Methods: We scheduled 11 healthy men to repeat 20-minute nap trials separated by 40-minute periods of enforced wakefulness across a period of 3 days. At the end of the nap trial, each participant answered questions regarding the formal aspects of his dream experiences during the nap trial, using the structured interviews.

Results: We obtained a total of 172 dream reports after naps containing REM sleep (REM naps) and 563 after naps consisting of only NREM sleep (NREM naps). Dream reports from NREM naps were less remarkable in quantity, vividness, and emotion than those from REM naps and were obtained more frequently during the morning hours when the occurrences of REM sleep were highest.

Conclusions: These results suggest that the polysomnographic manifestations of REM sleep are not required for dream experiences but that the mechanisms driving REM sleep alter experiences during NREM sleep in the morning. A subcortical activation similar to REM sleep may occur in human NREM sleep during the morning when REM sleep is most likely to occur, resulting in dream experiences during NREM sleep.

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INTRODUCTION

IN 1953, ASERINSKY AND KLEITMAN DISCOVERED HUMAN RAPID EYE MOVEMENT (REM) SLEEP AND DOCUMENTED THAT DREAM REPORTS WERE OBTAINED MOST FREQUENTLY WHEN SUBJECTS WERE AWAKENED FROM REM SLEEP.[1] Thereafter, many scientists conducted studies on dream and REM sleep and found a robust association between electrophysiologic phenomena and subjective experiences during REM sleep.[2-7] This well-documented association has led to the conclusion that dream experiences are psychological manifestations generated by the neural system controlling REM sleep,[8,9] yielding many innovative findings on the mind-body relationship. In contrast, researchers have also recorded dream reports from subjects upon awakening from non-REM (NREM) sleep; though the association has been shown to be weaker in comparison with that of dream reports upon awakening from REM sleep.[10-15]

Many human studies, together with some animal studies on REM sleep, have proposed a neural system responsible for human dream experiences. In the early pioneering studies on human REM sleep, researchers focused on the relationship between eye movements and visual experiences during REM sleep and postulated that REM and concomitant activation of the visual system of the brain account for human dream experiences.[2-4] Recent neurobiologic findings obtained from animal studies have led to the current understanding that phasic signals arising from the pons and impinging upon the cortex during REM sleep might give rise to dream experiences.[8,9]

Some studies have focused on dream experiences during NREM sleep; however, no documented findings have yet afforded an understanding of the mechanisms of dreaming during NREM sleep. Rather, researchers have generally made the assumption that dream reports upon awakening from NREM sleep may be a consequence of recalling dream experiences from the preceding REM sleep rather than indicate the existence of NREM-specific dream experiences.[7,16-17] However, only a few studies have aimed to determine whether dream reports after NREM sleep are derived from residuals of memory from the preceding REM sleep or actually arise from another type of dream experience during NREM sleep.

This may be due to the methodologic limitations of the conventional intermittent awakening method, in which subjects under all-night polysomnography are awakened several times upon reaching the target sleep stage and asked about their dream experiences. However, investigation of NREM dreaming may require a method in which subjects enter a sleep period only consisting of NREM sleep separated by a sufficient period of wakefulness to exclude the influence of the preceding REM sleep. These prerequisites, however, have not been satisfied in most previous studies except for a study that examined dream experiences during a short and discrete sleep period.[18]

In the study reported here, we used a repeated-nap trial, in which 20-minute sleep periods separated by 40 minutes of enforced wakefulness were repeated for 78 hours to allow the

Disclosure Statement

This is not an industry supported study. Drs. Suzuki, Uchiyama, Tagaya, Ozaki, Kuriyama, Aritake, Shibui, Tan, Kamei, and Kuga have indicated no financial conflicts of interest.

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measurement of various REM-NREM parameters in association with reported dream experiences.

METHODS

Participants

Eleven healthy male volunteers aged 20 to 26 years (mean = 22.4, SD = 2.1) participated in the present study. They did not have any known sleep, physical, or psychiatric disorders or any history of using psychoactive drugs. The study protocol was approved by the Intramural Research Board of the National Center of Neurology and Psychiatry, and each participant gave his informed consent after the nature, purpose, and possible risks of the experiment had been explained in detail.

All the participants were instructed to abstain from alcohol, caffeine, and napping for a week prior to the laboratory experiment. They were asked to keep sleep logs for 2 weeks and to wear wrist activity recorders (Actiwatch-L; Mini-Mitter, Bend, Ore) for the second week. Consistent sleep-diary and activity data were obtained from all the participants.

Study Design

Each participant took part in a 4-day experimental laboratory session. The participants arrived at the laboratory at 11:00 AM and consumed a 700- to 800-kcal lunch at 12:00 PM on day 1. Electrodes for standard polysomnography were attached between 1:00 PM and 3:00 PM; electroencephalogram (C3–A2, C4–A1, O1–A2), electrooculogram (left and right), chin-surface electromyogram, and electrocardiogram electrodes were used. The light level was set at 200 lux from 11:00 AM to 4:00 PM.

The participants entered a repeated nap trial at 4:00 PM on day 1. This involved 20-minute nap trials every 60 minutes, with standard polysomnographic recordings performed as participants lay recumbent on a bed in a dark (< 0.1 lux) sound-attenuated room, and a 40-minute period of enforced wakefulness on a semi-upright sofa under conditions of dim light (< 8 lux). During the 40-minute periods, participants were kept awake and monitored closely by experimenters. At the end of the nap trial, each participant was awoken gently. While remaining in a recumbent position, each participant answered questions regarding the formal aspects of his dream experiences during the nap trial, using the structured interview form described below, and thereafter left the bed. This cycle was repeated 78 times until 10:00 PM on day 4 (Figure 1). In the repeated nap trial, saliva samples were taken every 60 minutes during the last 5 minutes of the 40-minute period using saliva collection tubes (Bühlmann Laboratories AG, Schönenbuch, Switzerland). During the repeated nap trial, the room temperature and humidity were controlled at 24.0°C ± 0.5°C and 60% ± 5%, respectively. Participants consumed a 150-kcal snack and 200 mL of water every 2 hours.

Measures

Polysomnographic Measures

Polysomnograms obtained during the nap trials were scored in epochs of 30 seconds according to standard criteria.[19] A nap trial that contained stage REM was defined as a *REM nap*, whereas a nap trial containing no stage REM but NREM stages was defined as a *NREM nap*. Nap trials not containing any sleep stages were excluded from further analyses. The summed duration of NREM sleep stages (stage 1, 2, 3, and 4) and stage REM in the nap trial were termed *NREM duration* and *REM duration*, respectively.

Saliva Melatonin

Saliva samples were immediately refrigerated at -30°C for later analysis of melatonin concentration. Saliva melatonin was measured with a highly specific direct double-antibody radioimmunoassay kit (Saliva Melatonin RIA kit, Bühlmann Laboratories AG).[20] The time point where the saliva melatonin level crossed 3.3 pg/mL was defined as the dim-light melatonin onset, as outlined by Campbell and Murphy.[21] Dim-light melatonin onset was used for determining relative clock time as described below.

Dream Report Questionnaire

A structured questionnaire was developed to investigate the formal aspects of dreaming, such as dream duration and quality. We did not ask the participants about detailed dream content at a given nap trial because this would have influenced dream reports at successive nap trials. The questionnaire contained the following questions:

Q1. "How much did you dream?" (0: none, 1: little, 2: a moderate amount, 3: a lot)

When the reply to Q1 was 0, Q2-4 were not asked. Otherwise, Q1 was followed by Q2-4.

Q2. "How vivid was the dream?" (0: not vivid at all, 1: rather vivid, 2: moderately vivid, 3: very vivid)

Q3. "How pleasant was the dream?" (0: not pleasant at all, 1: rather pleasant, 2: very pleasant)

Q4. "How unpleasant was the dream?" (0: not unpleasant at all, 1: rather unpleasant, 2: very unpleasant)

The participant's responses to Q1 were averaged separately for REM naps and NREM naps (dream duration). Likewise, the participant's mean scores for Q2, Q3, and Q4 (vividness, pleasantness, and unpleasantness) after REM and NREM naps were calculated. Participants were considered to have had a dream experience if their response to Q1 was 2 or 3. Data from 4 nap trials were eliminated because of difficulties with electroencephalogram recordings.

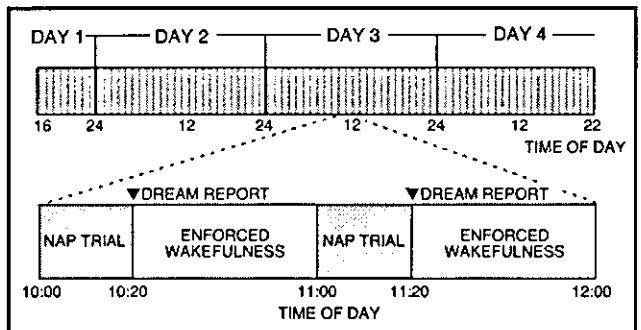

Figure 1—Experimental schedule. Participants followed a 60-minute sleep-wake cycle consisting of a 20-minute nap trial, followed by 40 minutes of enforced wakefulness. Standard polysomnographic recordings were made. This schedule was repeated for 78 hours from 4:00 PM on day 1 to 10:00 PM on day 4. At the end of each nap trial, a dream report was obtained using a structured interview.

Temporal Fluctuation

The data series obtained from the 78-hour experiment was time-locked to the dim-light melatonin onset, to which we designated a relative clock time of 10:00 PM. Thereby, we obtained standardized 72-hour data on all the participants. In this analysis, data obtained from only 9 participants were used, as melatonin measurements from 2 participants were not available because of lack of saliva samples.

Statistical Analyses

Paired *t* tests were performed to compare REM naps and NREM naps on measures of dream duration, vividness, pleasantness, and unpleasantness. For correlation analysis, we calculated Spearman correlation coefficients.

For analysis of temporal fluctuation, we used 72-hour data on REM duration, NREM duration, and dream duration. These were evaluated using 2-way repeated-measure analysis of variance (day and time of day) with a Huynh-Feldt epsilon correction. When a day effect was not observed, the 72-hour data was averaged into 2-hour bins in order to observe the 24-hour fluctuation of these measurements. For all the statistical analyses, the level of significance was set at $P < .05$. Statistical analyses were performed using StatView v5.0 (SAS Institute Inc., Cary, NC) and Super ANOVA (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Overview

Administering the structured questionnaire just after each 20-minute nap trial, we obtained a total of 854 dream reports from 11 participants. We excluded those reports obtained after nap trials without any sleep stages (n = 119); all the 30-second epochs were scored as stage wake. Finally, we had a total of 172 dream reports after REM naps and 563 after NREM naps.

Dream Reports from REM and NREM Naps

First, we compared the formal aspects of dream experiences between the REM naps and the NREM naps. Dream experiences were reported for 51.2% of the REM naps and 17.9% of the NREM naps. Dream experiences were more frequently found in the REM naps compared with the NREM naps (χ^2 (1) = 76.13, $P < .0001$). It was noted that dream experiences did occur during naps consisting of NREM sleep only. When dream experiences were averaged across participants, dream experiences were also more frequent in the REM naps than in the NREM naps (REM: 47.7%, NREM: 18.5%, $df = 10$, $t = -4.58$, $P = .0010$).

Table 1—Dream reports of NREM and REM naps

Dream report scores (range)	NREM NAP	REM NAP	*P* value
Dream duration (0-3)	0.47 ± 0.46	1.22 ± 0.76	.0003
Vividness (0-3)	0.46 ± 0.46	1.41 ± 0.93	.0003
Pleasantness (0-2)	0.41 ± 0.40	0.94 ± 0.47	.0057
Unpleasantness (0-2)	0.12 ± 0.22	0.41 ± 0.46	.0670

NREM refers to non-rapid eye movement; REM, rapid eye movement.

Subjective dream duration during the REM naps was significantly higher than that during the NREM naps ($df = 10$, $t = -5.41$, $P = .0003$) (Table 1). Dream experiences during the REM naps were more vivid and more pleasant than those during the NREM naps ($df = 10$, $t = -3.73$, $P = .0047$; and $df = 8$, $t = -3.74$, $P = .0057$, respectively). The level of dream unpleasantness during the REM naps was higher than that during the NREM naps, but this difference was not significant ($df = 8$, $t = -2.118$, $P = .067$). Dream experiences during NREM sleep seemed to be less

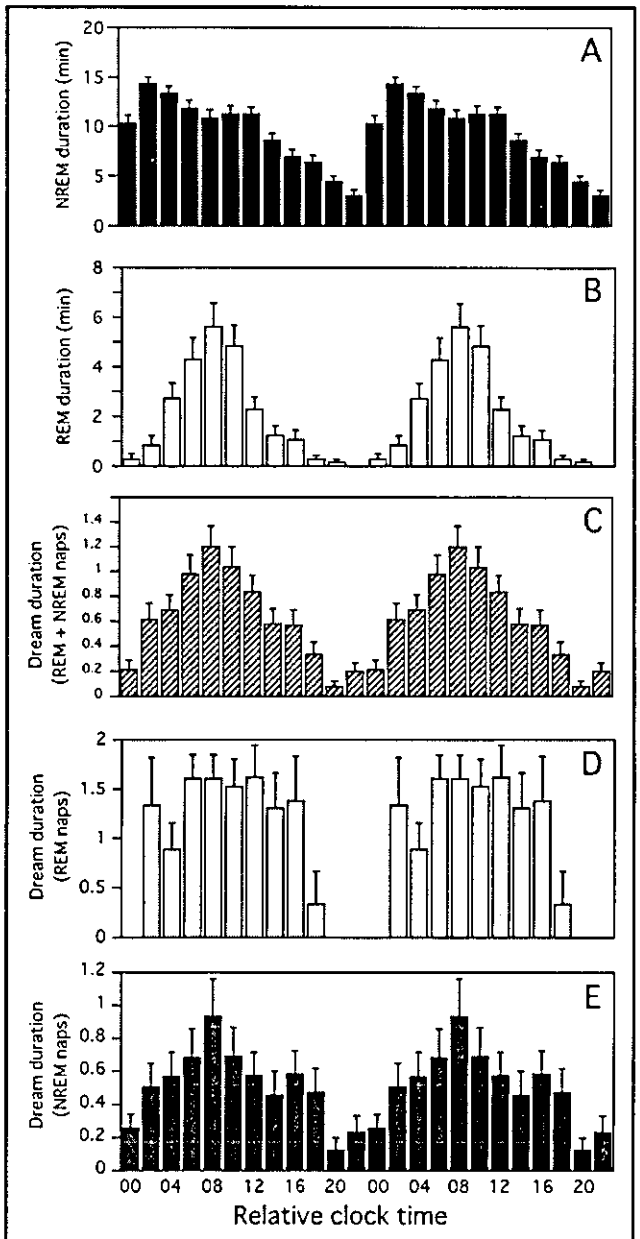

Figure 2—Temporal fluctuations in sleep duration and dream duration. All data are depicted in double-plot style and time-locked to the onset of melatonin release at a relative clock time of 10:00 PM. The peak of rapid eye movement (REM) duration (B) follows that of non-REM (NREM) duration (A) and coincides with that of the dream duration of REM + NREM naps (C). The dream duration of REM naps (D) shows no clear peak, whereas that of NREM naps (E) has a clear peak that coincides with the peak of REM duration but not with NREM duration.

remarkable in quantity, vividness, and emotion than those during REM sleep.

Temporal Fluctuations

Since it has long been recognized that REM sleep occurrence shows temporal characteristics across the day,[22] we then investigated the temporal fluctuations in NREM and REM duration and dream quantities across 2 days (72 hours). Two-way repeated analyses of variance (day and time of day) revealed a significant time-of-day effect on these 3 measures (NREM: $F = 8.64$, $P < .0001$, REM: $F = 3.92$, $P = .0055$, dream duration: $F = 2.75$, $P = .025$) but no day effect nor interaction, such that the 24-hour fluctuation curves did not differ in shape across the 3 days. We then averaged the data across the 72-hour period to obtain 24-hour fluctuation curves.

NREM duration increased sharply from 12:00 AM to 2:00 AM, where a clear peak on the fluctuation curve was evident (Figure 2, A). Thereafter, NREM duration showed a gradual decrease towards the late evening hours with a small peak at 12:00 PM. NREM duration was shortest during the late evening hours (10:00 PM). In contrast, REM duration gradually increased in the midnight and morning hours, showing a maximum at 8:00 AM and a gradual decrease thereafter (Figure 2, B). Dream duration of REM plus NREM naps (the mean value averaged across all REM and NREM reports) increased in the midnight and morning hours and reached a peak at 8:00 AM, followed by a gradual decrease towards the late evening hours (Figure 2, C). The peak in dream duration of REM plus NREM naps coincided with that of REM duration but not with that of NREM duration, which appeared 6 hours earlier. The fluctuation curve of dream duration of REM plus NREM naps appeared similar to that of REM duration in shape, but its distribution was wider.

Next, we constructed figures of the temporal fluctuations in dream duration with respect to the REM and NREM naps, separately. The dream duration of REM naps was high during the period 6:00 AM to 12:00 PM and showed no clear peak (Figure 2, D). In contrast, there was a clear peak at 8:00 AM with the dream duration of NREM naps (Figure 2, E). Surprisingly, this peak coincided with the peak in REM duration but not with that of NREM duration. Correlation analysis between REM duration and the dream duration of NREM naps revealed that REM duration explained a remarkable 75% of the temporal fluctuation of the dream duration of NREM naps ($r = .87$, $n = 11$, $P < .0001$, $r^2 = .75$).

DISCUSSION

In the present study, we conducted 20-minute nap trials every hour for 78 hours (repeated nap trial), calculated sleep indexes during the nap trials, and obtained dream reports at the end of each nap trial. Using the repeated nap trial, we compared the quantity and quality of dream experiences between REM sleep and NREM sleep and investigated the temporal fluctuations in dream duration.

Use of the repeated nap trial allowed us to determine that dreams reported at the end of a given nap trial were experienced exclusively during the period of that nap trial. In contrast, use of the conventional study methods—in which investigators typically monitor an all-night polysomnogram and wake subjects at targeted sleep stages—fail to determine when reported dreams were actually experienced. We found that the quantity, vividness, and emotional changes in dreams occurring during the REM naps were more marked than those experienced during the NREM naps and that dream experiences also occurred more frequently in REM naps than in NREM naps, the figures being comparable to those of previous studies conducted using the conventional paradigm.[13]

It was noted that 17.9% of the NREM naps were associated with dream experiences, suggesting that participants did experience dreams during nap trials consisting of NREM sleep only. Researchers conducting studies using the conventional paradigm have postulated that such NREM dreams do occur. However, no study has yet confirmed in which sleep stage dreams reported by subjects actually occurred, except for a study by Takeuchi et al,[18] in which experiences during short naps (about 10 minutes) were examined. Their results—that experiences during NREM sleep were strongly influenced by the duration of wakefulness contaminated in the nap—may suggest that 10 minutes are not enough to assess experiences during NREM sleep accurately. The fact that human dream experiences can occur during a sleep period without REM was first properly confirmed systematically in the present study, due to the advantage of using a 20-minute repeated nap trial.

Temporal Fluctuations of Dreaming

Dream duration showed an apparent peak in the morning hours, coinciding with peaks in REM duration but not NREM duration. It seems that humans are most likely to experience dreams in certain morning hours of the day.

When a differential analysis was undertaken on dream duration in the REM naps and NREM naps, the NREM curve showed a clear peak in the morning hours, whereas no clear peak was observed in the curve representing the REM naps. The present finding that dream duration in the NREM naps fluctuated across the day in parallel with REM duration suggests that human dream experiences in NREM sleep are strongly influenced by the REM sleep-generating mechanism. Prior animal studies on REM sleep and pontogeniculooccipital activity may provide an explanation for this assumption. In a study by Callaway et al, pontogeniculooccipital activity, which is generated in the brain stem and characterizes animal REM sleep, was observed to also occur in NREM sleep preceding the REM sleep period, even when the cortical electroencephalogram displayed characteristics of NREM patterns such as slow waves.[23] Pontogeniculooccipital activity observed in animal REM sleep is considered to be the most robust factor activating the visual cortexes and generating dream experiences.[9] Similar activation of the visual cortexes may occur in human NREM sleep, especially during the morning hours when REM sleep is most likely to occur, resulting in dream experiences during NREM sleep.

There is a long-standing controversy surrounding the existence of dream experiences during NREM sleep. Some researchers have assumed that dreams recalled upon awakening from NREM sleep are a consequence of the mnemonic effects of prior REM sleep.[7,16] Others have postulated that NREM dreams are different from REM dreams, possibly because the loci of REM and NREM sleep generation are exclusively independent.[24-26] Our results appear to support both early suggestions that NREM dreaming

may be due to activation of REM-related processes during NREM-REM transition periods[16,27] and the more recent suggestion that REM sleep processes can operate covertly to produce NREM dreaming at any time.[17] The results obtained from the repeated nap trial reported here provide understandings of the relationship between dream experiences and NREM sleep. The remarkable correlation we observed between REM duration and NREM dream duration suggests that increases in REM sleep propensity may lead to increased dream production even during NREM sleep.

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Brief Report

Deactivation by Benzodiazepine of the Basal Forebrain and Amygdala in Normal Humans During Sleep: A Placebo-Controlled [^{15}O]H_2O PET Study

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Objective: The authors' goal was to identify differences in regional brain activity between physiological and benzodiazepine-induced sleep to clarify the brain structures involved in the drug's hypnotic effect.

Method: Using positron emission tomography, they compared regional cerebral blood flow during non-REM sleep in nine volunteers treated with placebo or triazolam, a short-acting benzodiazepine, in a double-blind, crossover design.

Results: Blood flow in the basal forebrain and amygdaloid complexes was lower during non-REM sleep when subjects were given triazolam than when they were given placebo.

Conclusions: The hypnotic effect of the benzodiazepines may be mediated mainly by deactivation of the forebrain control system for wakefulness and also by the anxiolytic effect induced by deactivation of the emotional center.

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Benzodiazepine hypnotics, which have been used widely since the early 1960s, are known to act by agonistic modulation of γ-aminobutyric acid A ($GABA_A$) receptor subtypes. However, the pharmacological actions mediated by the $GABA_A$ receptor subtypes are still a matter of dispute (1). Therefore, many questions regarding the specific drug actions of the benzodiazepine hypnotics remain to be answered, including the question of which neuroanatomical sites are affected by these drugs.

Positron emission tomography (PET) has been used effectively to visualize the functional neuroanatomy of human sleep, and oxygen-15 water ([^{15}O]H_2O) PET, which is well suited to the investigation of sleep states of relatively short duration, has revealed changes in regional cerebral blood flow (rCBF) associated with human non-REM and REM sleep (2). The effects on human sleep of hypnotics acting on the $GABA_A$ receptor, including the newly developed nonbenzodiazepines, have also been explored by using PET (3). Unfortunately, previous studies failed to detect specific neuroanatomical sites for the actions of these hypnotics during sleep, possibly because of methodological problems. Therefore, we conducted a double-blind, crossover study of [^{15}O]H_2O PET with statistical parametric mapping to compare rCBF during human non-REM sleep after administration of triazolam or placebo.

Method

Fifteen healthy, right-handed, male university students (mean age=21.3 years, SD=1.0, range=20–23) served as study subjects. All gave written informed consent before participating in the study, which was approved by the Intramural Research Board of the National Center of Neurology and Psychiatry.

A maximum of eight intravenous injections of [^{15}O]H_2O were given to each subject during periods of wakefulness and light and deep non-REM sleep under continuous polygraphic monitoring on each experimental night of triazolam or placebo administration. The experiments were separated by a 1-week interval and did not include sleep deprivation. On the night of the experiment, electrodes were attached for polygraphy, and each subject lay face up on a scanner couch, with the head stabilized by an individually molded thermoplastic face mask secured to a plastic headholder. The headholder was fixed on the end of the scanner couch so that the head was protruding fully into the scanner field of view; the head was angled so that each subject's canthomeatal line was parallel to axial planes of the PET scanner.

After two PET scans for wakefulness were obtained in the eye-closed condition, the subject took a capsule containing either 0.25 mg triazolam or placebo at 10:00 p.m. The lights were turned out at 10:30 p.m., and three scans for light non-REM sleep (stage 2 sleep) and three for deep non-REM sleep (stages 3 and 4 sleep, slow wave sleep) were performed between 11:00 p.m. and 2:00 a.m., when the polygrams showed typical patterns for these sleep stages.

EEGs were recorded from disc electrodes placed at F3, F4, C3, C4, Fz, and Cz; A1 plus A2 were used for reference. Monopolar electro-oculograms were recorded from both canthi, and bipolar

electromyograms were recorded from the chin. Details of the polygraphic methodology are the same as in our previous study (4). Sleep stage scoring was carried out according to the standardized sleep manual of Rechtschaffen and Kales (5). Final evaluation of sleep stage scoring for each 90-second period during PET scanning was confirmed later by using C3 recording.

PET data were acquired with the use of a Siemens ECAT EXACT HR 961 scanner in the three-dimensional mode. The camera, having an axial field of view of 150 mm, acquired data simultaneously from 47 consecutive axial planes, which cover the whole brain, including the cerebrum, cerebellum, and brainstem. A spatial resolution of 3.8×3.8×4.7 mm of full width at half maximum was obtained after back-projection and filtering. The reconstructed image was displayed on a 128×128×47-voxel matrix (voxel size=1.732×1.732×3.125 mm). Transmission scanning was carried out before acquisition of the emission data by using a retractable, rotating $^{68}Ga/^{68}Ge$ source with three rods.

For each PET scan, an intravenous bolus of 7-mCi $[^{15}O]H_2O$ was automatically flushed over 15 seconds. Scanning was begun manually 1 second after the initial rise in head counts and was continued for 90 seconds. Arterial blood was sampled automatically throughout the scanning period with a flow-through radioactivity detector. Absolute CBF was quantified by using the autoradiographic technique (6, 7). Details of the PET data analysis are the same as in our previous study (8).

After the appropriate design matrix was specified, estimates of the subject and condition were determined according to a general linear model at each and every voxel. Parameter estimates were compared by using linear contrasts. The contrast of interest in this article was the main effect of the drug during non-REM sleep. These analyses generated statistical parametric maps that were subsequently transformed to the unit normal distribution. The exact level of significance of volumes of difference between conditions was characterized by peak amplitude. Clusters of voxels that had a peak z score greater than 3.09 (threshold $p<0.001$) were considered to show significant difference. A corrected p value of 0.05 was used as a statistical cluster threshold.

Results

We analyzed the PET data of nine of the 15 subjects, who provided us with complete sets of data during periods of wakefulness and light and deep non-REM sleep on the nights of triazolam and placebo administration. One-way analysis of variance showed no significant difference in the absolute values for global CBF during light or deep non-REM sleep between the placebo and triazolam conditions. Mean absolute global CBF during light non-REM sleep with placebo was 30.4 ml/100 g per minute (SD=5.1), and that with triazolam was 28.8 ml/100 g per minute (SD=4.6) ($F=0.51$, $p=0.49$). Mean absolute global CBF during deep non-REM sleep with placebo was 30.6 ml/100 g per minute (SD=3.2), and that with triazolam was 29.4 ml/100 g per minute (SD=3.1) ($F=0.64$, $p=0.44$).

When there are no differences in the absolute values for global CBF, comparison of normalized values for regional CBF is often more sensitive for detection of differences than comparison of absolute values and reflects real changes in rCBF (2, 8). Therefore, normalized rCBF values obtained for non-REM sleep with placebo were compared with those obtained with triazolam by analysis of covariance for the nine subjects who provided complete sets of data.

TABLE 1. Brain Regions Showing Significantly Lower Blood Flow in Nine Normal Volunteers During Non-REM Sleep After Triazolam Administration Than After Placebo Administration

Region	Brodmann's Area	Coordinates[a] x	y	z	z Score
Amygdaloid complex					
Left		−32	0	−18	4.68
Right		28	−4	−22	3.47
Caudal orbital basal forebrain					
Left		−18	20	−14	4.40
Right		22	10	−18	6.11
Basal forebrain					
Left		−14	0	−10	4.08
Right		10	0	−4	5.86
Anterior cingulate gyrus					
Left	24	−6	−4	36	3.68
Right	32	4	40	6	3.72
Posterior cingulate gyrus					
Left	23	−4	−16	28	3.33
Right	23	4	−26	26	3.65
Left insula	13	−40	−10	−4	3.29
Left prefrontal cortex superior frontal gyrus	10	−20	60	−8	4.62
Left precentral gyrus	44	−44	18	8	4.39
Left superior temporal gyrus	38	−50	20	−14	4.92
	22	−48	−14	8	4.02
Left superior parietal gyrus	7	−36	−66	48	3.75

[a] Coordinates are defined in the stereotactic space of Talairach and Tournoux (9), relative to the anterior commissure: x is the lateral distance from the midline (positive=right), y is the anteroposterior distance from the anterior commissure (positive=anterior), and z is the rostrocaudal distance from the bicommissural plane (positive=rostral). Significance level is employed at a height threshold of $p=0.001$, by reference to unit normal distribution ($z=3.09$), and at a threshold of corrected $p<0.05$.

Blood flow was lower in the basal forebrain, amygdaloid complexes, anterior (Brodmann's area 32, 24) and posterior (Brodmann's area 23) cingulate gyri, and the left neocortical regions, including the superior temporal gyrus (Brodmann's area 38, 22), precentral gyrus (Brodmann's area 44), superior frontal gyrus (prefrontal cortex) (Brodmann's area 10), superior parietal gyrus (Brodmann's area 7), and insula (Brodmann's area 13), during non-REM sleep when triazolam was given than when placebo was given (Table 1 and Figure 1). There was no significant increase in rCBF of any region during non-REM sleep under triazolam administration in comparison to that under placebo administration.

Discussion

Using functional neuroanatomical PET, we found that the basal forebrain and the amygdaloid complexes of human subjects showed deactivation during non-REM sleep after triazolam treatment. Wakefulness is known to be maintained by multiple neuronal populations spread out from the metencephalon to the diencephalon, the mesencephalic reticular formation, posterior and posterolateral hypothalamus, and basal forebrain cycle (10). Our data show that triazolam has its main impact on the basal fore-