Sleep-dependent surges in growth hormone do not contribute to sleep-dependent memory consolidation

Steffen Gais*, Philipp Hüllmann, Manfred Hallschmid, Jan Born

Department of Neuroendocrinology, University of Lübeck, Ratzeburger Allee 160, Hs. 23a, 23538 Lübeck, Germany

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Summary In the search for the mechanisms that mediate the effects of sleep on the consolidation of memories, growth hormone (GH) recently became of interest, because in humans it is released mainly during slow-wave sleep (SWS), a period of enhanced declarative memory consolidation. In addition, recent studies showed that GH is involved in proper memory function in GH deficient and elderly humans and this effect has been linked to regulatory influences of GH on hippocampal NMDA receptors. Here, we blocked GH secretion by intravenous infusion of somatostatin in healthy young subjects during the first 3 h of sleep, which contain mainly SWS. Declarative and procedural memory consolidation was tested across this period, using a word pair association task and a mirror tracing task, respectively. Although GH was effectively suppressed, memory performance as well as sleep were entirely unaffected by this suppression. Whereas GH may in the long run generally support brain systems required for maintaining proper memory function, our data exclude a necessary contribution of the nocturnal surge in pituitary GH secretion to the acute processing and formation of specific memories during sleep.

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1. Introduction

In recent years, a number of studies confirmed the notion that sleep enhances memory consolidation in both the declarative and the procedural memory systems (Gais and Born, 2004a; Walker and Stickgold, 2004). However, the mechanisms possibly mediating this function of sleep, like the various changes in brain activity and endocrine secretion, are still poorly understood. Most theories on how sleep improves memory consolidation come from animal studies, which investigated the neural brain electrical activity that presumably underlies information processing during sleep. The contribution of endocrine activity related to sleeping on the other hand, has not received much attention, although there are good reasons to assume that hormones are involved in modulating memory systems (Payne et al., 2005).

Neuroendocrine activity during sleep is very different from that of wakefulness. Hormone
secretion and activity of neuromodulators change radically at the transition form wakefulness to sleep and between non-rapid eye movement (NREM) and REM sleep stages. Characteristic features of nocturnal slow-wave sleep (SWS) are the circadian nadir of cortisol secretion, the high release of growth hormone (GH) and the cessation of neuromodulatory cholinergic activity (Born and Fehm, 1998; Hasselmo, 1999). Some of these changes have already been implicated as mediators in the relationship between sleep and memory. The suppression of cortisol release during the first hours of nocturnal sleep was one of the first characteristics of sleep shown to enhance sleep related declarative memory consolidation (Plihal and Born, 1999). Lack of cholinergic modulatory activity during SWS, which supposedly switches the hippocampal memory system into a 'replay' mode (Hasselmo, 1999), also proved a necessary condition for declarative memory consolidation during sleep (Gais and Born, 2004b).

Growth hormone is a peptide hormone that is secreted principally from the anterior pituitary. Via hypothalamic inputs its release is mainly stimulated by GH-releasing hormone and inhibited by somatostatin. GH has been related in previous studies to sleep and to memory function. GH in adult humans is secreted mainly during the first part of nocturnal sleep in close association with the intense periods of SWS predominating this part of the night (Born and Fehm, 1998). This phase of sleep is also known to contribute to declarative memory consolidation (Plihal and Born, 1997). Several authors have proposed that GH is also involved in memory function, e.g. by inducing gene expression for glutamatergic NMDA receptors in the hippocampus, a process assumed to underlie declarative memory function (Nyberg, 2000; Le Greves et al., 2002). GH prevents neuronal loss in the hippocampus of old rats (Azcoitia et al., 2005), and, in patients with GH deficiency, replacement of GH leads to enhancement of short- and long-term declarative memory performance (Arwert et al., 2005). Together, such findings led Payne et al. (2005) to suggest that GH release during early SWS-rich sleep may contribute to the enhanced memory consolidation taking place during this part of sleep. The aim of the present study was to investigate this possible relationship between sleep-dependent GH secretion and memory consolidation during sleep. To achieve this aim, healthy subjects were administered intravenous somatostatin, which consistently suppresses GH secretion, but does not pass the blood-brain barrier and, thus, is not active centrally (Meisenberg and Simmons, 1983).

2. Methods

2.1. Subjects

Fifteen healthy young males (19-30 years) participated in the experiments. All were non-smokers, had no acute or chronic disease and were not taking any medication at the time of the experiments. They did not report any sleep disturbances and no disruption of their sleep-wake cycle during the six weeks prior to the experiments. Subjects were accustomed to sleeping under laboratory conditions during one night before the experiments. One subject was removed from analysis because somatostatin did not lead to any GH suppression in his case. Experiments were approved by the Ethics Committee of the University of Lübeck, and all subjects gave written informed consent.

2.2. Design and procedure

Each subject participated in two experimental conditions according to a randomized and balanced cross-over design. In one condition, subjects received 6 µg per kg bodyweight of somatostatin (Somatostatin 3 mg Curamed®, half-life in plasma 1-3 min) dissolved in 50 ml of isotonic saline solution as intravenous infusion over 3 h. In the other condition, subjects received only 50 ml of isotonic saline solution. Subjects and experimenter were blind with regard to the experimental condition. The two sessions for a subject were separated by an interval of at least two weeks. In both nights, subjects came to the laboratory in the evening at 2100 h. Two forearm venous catheters were placed for blood sampling and substance administration. Then electrodes for two channel EEG (C3 and C4), horizontal and vertical EOG and EMG recording were applied. From 2200 to 2230 h subjects memorized a list of word pairs for declarative memory testing and learned a procedural memory task. Afterwards subjects went to bed and lights were turned off at 2300 h. From sleep onset, substance was administered for 3 h, then subjects were woken. Thirty minutes after awakening, retrieval of word pair lists and level of procedural skill performance was tested. In addition, after learning and retrieval testing, simple reaction times were measured as an indicator of vigilance. For
this, subjects had during 35 trials to press as fast as possible a button in response to a stimulus on a computer screen. The task also included five no-go trials. In the end, a questionnaire was given to assess the subjects’ mood, wakefulness and calmness.

2.3. Tasks

Two different memory tasks were used which had proved to be sensitive to the effects of sleep in similar previous studies (e.g. Plihal and Born, 1997; Gais and Born, 2004b). Declarative memory was tested using a word pair learning task, which required subjects to learn lists of word pairs and to name the second word of each pair when presented with the first one at recall testing (Plihal and Born, 1997). Lists consisted of 40 pairs of semantically related words and were randomized on each presentation. Pairs were presented for 5 s each. After learning, immediate recall was tested and the correct answer was presented as a feedback for 2 s. If a criterion of 60% correct responses was not reached, the recall procedure was repeated.

To test procedural memory, subjects performed a mirror tracing task (Plihal and Born, 1997). This task required the subjects to trace several figures which they could see only in a mirror. The time needed for completion of the figures (speed) and the number of deviations from the prescribed 0.8 cm wide path (accuracy) were recorded. Subjects were instructed to trace the lines of the figures as fast and as accurately as possible. Before learning the actual figures, subjects trained a simple star-shaped figure until they could draw it in less than 30 s with less than 12 errors. The results are given as the average performance on the six figures. Parallel versions of the tasks were used on the subject’s two testing occasions.

2.4. Hormone assessment, sleep scoring and subjective measures

Blood samples were taken after learning and retrieval testing as well as hourly during sleep, beginning 30 min after sleep onset. Blood glucose was determined immediately after blood sampling with a HemoCue Glucose System (HemoCue GmbH, Großostheim, Germany). Samples for determination of GH, cortisol, insulin and IGF-1 were centrifuged and frozen immediately for later analysis. All hormones were assessed from serum by competitive chemoluminescence immunoassay with an Immulite System (DPC-Biermann GmbH, Bad Nauheim, Germany). Sleepiness, mood and subjective sleep quality were measured before and after sleep on 5-point Likert scales.

3. Results

The effects of somatostatin administration on systemic hormone levels and blood glucose are summarized in Fig. 1. As expected, somatostatin administration induced a substantial reduction in GH secretion and insulin concentrations to levels close to the sensitivity of the assay ($F=3.6$, $p=0.01$ and $F=11.8$, $p<0.001$, respectively, for substance×time ANOVA interaction). In addition, slight changes in cortisol and a marginal decrease in glucose were seen in response to somatostatin administration ($F=2.9$, $p=0.04$ and $F=2.5$, $p=0.06$, respectively). IGF-1 did not differ between the somatostatin and placebo conditions ($F=0.3$, $p>0.60$ for main effect of substance; $F=0.8$, $p>0.5$ for substance×time interaction).

Sleep patterns were similar in both treatment conditions. Sleep stages were distributed as follows (% of total sleep time for the placebo and somatostatin conditions, respectively): awake 1.0±0.4% vs. 1.7±1.0%; stage 1 sleep 9.3±2.6% vs. 10.6±3.4%; stage 2 sleep 51.2±3.9% vs. 47.8±3.6%; slow wave sleep 27.9±3.8% vs. 28.6±3.8%; REM sleep 10.2±1.1% vs. 10.7±2.2%. ($p>0.4$, for all comparisons). Total sleep time was 178±4.3 min vs. 167±11.1 min ($p>0.15$).

Performance on both tasks of declarative and procedural memory remained unaffected by systemic somatostatin administration. Measures of declarative memory consolidation were practically identical in both conditions: the number of correctly remembered word pairs increased from learning before sleep to retrieval testing after sleep on average by 3.6±0.6 word pairs in the placebo condition, and by 3.4±0.7 word pairs in the somatostatin condition ($t=0.27$, $p=0.79$, Table 1). Procedural memory consolidation also showed no difference between conditions, with the increase in speed during mirror tracing from acquisition before sleep to retrieval testing after sleep averaging 6.4±2.3 s after placebo and 10.7±3.1 s after somatostatin ($t=1.0$, $p=0.33$). Changes in error counts across sleep also did not differ between the placebo ($−8.65±1.24$) and somatostatin conditions ($−8.26±2.0$, $t=−0.19$, $p=0.85$, see Table 1 for performance measures separately for learning and retrieval testing).

Reaction times, too, did not indicate differences in vigilance between conditions at retrieval testing.
Also, mood, subjective sleep quality and sleepiness were comparable at retrieval testing after sleep on both treatment conditions ($t = 0.03, p = 0.98$). Also, mood, subjective sleep quality and sleepiness were comparable at retrieval testing after sleep on both treatment conditions ($p > 0.5$ for all subjective scales).

4. Discussion

Although somatostatin was obviously effective in suppressing GH secretion, there was no effect at all

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of declarative and procedural memory tasks.</th>
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<tbody>
<tr>
<td>Condition</td>
<td>Acquisition</td>
</tr>
<tr>
<td>Word pairs remembered</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>Somatostatin</td>
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<tr>
<td>Mirror tracing speed</td>
<td>Placebo</td>
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<td></td>
<td>Somatostatin</td>
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<td>Mirror tracing errors</td>
<td>Placebo</td>
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<td>Somatostatin</td>
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Differences between placebo and somatostatin conditions are not significant.
on memory consolidation. This result strongly suggests that pituitary GH has no necessary function in the acute consolidation of declarative memories that takes place during the first hours of human sleep, when SWS is predominant. This result is unexpected with regard to the mounting evidence that GH is related to memory function: GH can enhance memory performance in GH deficient and elderly patients (van Dam et al., 2000), it prevents loss of neurons in the rat hippocampus (Azcuita et al., 2005) and it affects NMDA receptor transcription in the hippocampus (Le Greves et al., 2002). Moreover, an enhancement of long-term memory after GH was found in rats for one-trial avoidance conditioning (Schneider-Rivas et al., 1992). The effects of GH on memory have been linked to GH receptors in the hippocampus (Nyberg, 2000). A likely explanation is that the function of GH is related to memory functioning in general and to the maintenance of memory systems, thus to the acute processing of specific memory contents during sleep.

As has been shown in several previous studies using exactly the same design as the present experiment, the paired associate task is better remembered after a period of sleep containing large amounts of SWS than after periods of REM sleep or wakefulness (Plihal and Born, 1997; Gais and Born, 2004b). The present experiments did not include REM sleep and wake control groups, because during these time periods GH secretion is minimal. Consistent with previous studies, we did not find any changes in sleep after administration of somatostatin (Steiger et al., 1992). Apart from GH, somatostatin affects a number of other hormones, like TSH, glucagon, insulin and several gastrointestinal hormones. Our data cannot rule out an interaction of these different hormones with their consequences on memory cancelling each other out. However, it is very unlikely that the effects of different hormone systems affected by systemic somatostatin have the same size but opposite directions. The main parameters that are affected by somatostatin and are supposed to play a role in memory consolidation, GH and insulin, are both supposed to enhance memory performance (Nyberg, 2000; Benedict et al., 2004). Given the suppressive action of somatostatin on these two hormones, rather than cancelling out, an augmentation of the effects of somatostatin administration on memory would have been expected.

Although we could not show an influence of GH on acute memory consolidation during sleep, there are two probable ways how somatotrophic activity can influence memory. On one hand, some GH releasing factor might be involved in acute memory consolidation. On the other hand, previous studies suggest that GH or substances related to GH release like IGF-1 are involved in long-term maintenance of the memory system. While these possibilities remain to be explored, the results we present here exclude any necessary function of pituitary GH on the acute formation of memories during sleep.

References


