

Central Nervous Insulin Signaling in Sleep-Associated Memory Formation and Neuroendocrine Regulation

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The neurochemical underpinnings of sleep's contribution to the establishment and maintenance of memory traces are largely unexplored. Considering that intranasal insulin administration to the CNS improves memory functions in healthy and memory-impaired humans, we tested whether brain insulin signaling and sleep interact to enhance memory consolidation in healthy participants. We investigated the effect of intranasal insulin on sleep-associated neurophysiological and neuroendocrine parameters and memory consolidation in 16 men and 16 women (aged 18–30 years), who learned a declarative word-pair task and a procedural finger sequence tapping task in the evening before intranasal insulin (160 IU) or placebo administration and 8 h of nocturnal sleep. On the subsequent evening, they learned interfering word-pairs and a new finger sequence before retrieving the original memories. Insulin increased growth hormone concentrations in the first night-half and EEG delta power during the second 90 min of non-rapid-eye-movement sleep. Insulin treatment impaired the acquisition of new contents in both the declarative and procedural memory systems on the next day, whereas retrieval of original memories was unchanged. Results indicate that sleep-associated memory consolidation is not a primary mediator of insulin's acute memory-improving effect, but that the peptide acts on mechanisms that diminish the subsequent encoding of novel information. Thus, by inhibiting processes of active forgetting during sleep, central nervous insulin might reduce the interfering influence of encoding new information.

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INTRODUCTION

The consolidation of memory contents, ie, the strengthening and reprocessing of encoded information, has been shown to strongly rely on the brain's offline processing during sleep (Diekelmann and Born, 2010). It is assumed that neuronal ensembles involved in information encoding during wakefulness are reactivated during subsequent sleep, strengthening respective memory representations (Diekelmann and Born, 2010). This concept relies on findings in rodents that firing patterns of hippocampal and cortical neurons observed during task performance while the animal is awake re-emerge during non-rapid-eye-movement (NonREM) sleep (Ji and Wilson, 2007; Wilson and McNaughton, 1994) and that re-exposing human participants during slow wave sleep to cues associated with the prior learning experience enhances sleep-dependent memory consolidation

(Oudiette and Paller, 2013). Notwithstanding intense research efforts, the neurochemical mechanisms underlying sleep-dependent memory consolidation have only been partially unraveled (Abel *et al*, 2013). Sleep provides a unique neurochemical milieu, and fluctuations in plasticity-promoting neuromodulators like cortisol, acetylcholine, serotonin, and norepinephrine have been found to contribute to sleep-dependent memory consolidation (eg, Gais and Born, 2004; Groch *et al*, 2011; Plihal and Born, 1999; Rasch *et al*, 2009), which moreover is subject to a modulatory influence by glutamatergic and GABAergic transmission (eg, Feld *et al*, 2013a,b; Gais *et al*, 2008).

The pancreatic hormone insulin, a major factor in the maintenance of energy homeostasis, has turned out to be an important neuromodulator, regulating among others metabolic function (Bruning *et al*, 2000) and neuronal plasticity (Chiu and Cline, 2010). Insulin reaches the CNS via saturable transport mechanisms but does not appear to be released in large amounts within the brain (Gray *et al*, 2014), although indicators of local insulin production in the cerebral cortex have been found in animals (Molnar *et al*, 2014). Central nervous insulin receptors are expressed in high densities in structures like the olfactory bulb, the hypothalamus, and, notably, the hippocampal formation (Devaskar *et al*, 1994),

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which is essential for the initial formation and storage of declarative memory, ie, memory for episodes that is accessible to conscious recollection. Accordingly, central nervous insulin administration via the intranasal pathway (Born *et al*, 2002; Dhuria *et al*, 2010) has been repeatedly found to improve memory function in healthy subjects when delivered acutely (Benedict *et al*, 2008; Brunner *et al*, 2015) or for 8 weeks (Benedict *et al*, 2004, 2007) and, moreover, in patients suffering from mild cognitive impairments and early Alzheimer's disease (Craft *et al*, 2012; Reger *et al*, 2008a, for review see De Felice, 2013). Memory-improving effects of insulin have been suggested to be mediated via changes in hippocampal synaptic plasticity including long-term depression and potentiation (Moult and Harvey, 2008), with potential contributions from enhanced neuronal glucose utilization (Doyle *et al*, 1995). However, the exact preconditions and mechanisms of insulin-induced memory improvements have remained largely unexplored.

Considering that insulin interacts with neuroendocrine factors like cortisol (Benedict *et al*, 2004; Bohringer *et al*, 2008) and growth hormone (Gahete *et al*, 2013), which are secreted in a circadian/sleep-dependent manner, in the present study, we aimed to clarify whether brain insulin signaling contributes to sleep-dependent memory formation, and how central nervous insulin administration via the intranasal pathway affects sleep-associated neuroendocrine regulation. Since previous studies have indicated that men and women respond differently to insulin's central nervous effects (Benedict *et al*, 2008; Hallschmid *et al*, 2004; Krug *et al*, 2010), we investigated the impact of pre-sleep intranasal insulin administration in male and female participants.

MATERIALS AND METHODS

Participants

A total of 32 young healthy, non-smoking, native German speaking participants (16 males and 16 females) completed the study (for details, see Supplementary Methods), which was approved by the local ethics committee. Written informed consent was obtained from all participants before participation.

Study Design and Procedure

The experiment followed a balanced, double-blind, placebo-controlled, within-subject, crossover design. All participants took part in two experimental sessions, which were identical except for the intranasal administration of insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark) or placebo (insulin carrier solution). During participation, all the women were taking estrogen-dominant oral contraceptives. Experimental sessions were scheduled to be apart as close to 28 days as possible (men, median, 28 days, range 27–35 days; women, median, 28 days, range 25–28 days), ensuring that the women were tested during the same phase of contraceptive intake in both sessions (see Supplementary Methods for details). Sessions were performed in a balanced order, ie, half of the sample received first placebo and then the active agent, with the reversed order for the other half of the sample. The experimental procedure is summarized in

Figure 1a and described in detail in the Supplementary Methods. In brief, at 2120 hours of the first evening, participants memorized declarative (word-pairs) and procedural (finger sequence tapping) contents before receiving intranasal insulin (or placebo) via 16 0.1-ml puffs (8 per nostril) in 1-min intervals, amounting to a total dose of 1.6 ml insulin (160 IU) or placebo at 2220 hours. Subjects went to bed at 2300 hours for 8 h of sleep that was recorded polysomnographically. At 1800 hours on the subsequent evening they learned interfering contents before retrieval of the contents learned before sleep and of the interfering information. Throughout the session, blood was repeatedly sampled for the determination of relevant parameters and mood and vigilance were assessed.

Memory Tasks

Declarative memory was investigated with a word-pair interference paradigm (see Supplementary Methods for details). During the first evening, participants learned a list of 20 cue words associated with unrelated target words (A-B) up to a criterion of 90% correct responses. On the subsequent evening they learned 20 interfering associations of the original cue words with completely new, unrelated target words (A-C), again until a criterion of 90% correct answers was reached. Afterwards, the original and the new associations were retrieved. For procedural memory testing, a finger sequence tapping task was used (for details see Supplementary Methods). On the first evening, participants learned a 5-element finger sequence by tapping it on a keyboard during 12 30-s blocks. On the subsequent evening, the participants learned a different 5-element sequence. Afterwards, both sequences were tested independently during additional three blocks.

Blood Analyses

Blood glucose levels and circulating concentrations of growth hormone and insulin were determined at two time points before substance administration and repeatedly thereafter (see Supplementary Methods). To adjust for baseline concentrations, individual averages across the 2115 hours and 2215 hours baseline values were subtracted from post-administration concentrations. For display purposes, the resulting differences were referenced to a common baseline. Pairwise comparisons between conditions were performed for each sampling time point as well as for the peak in growth hormone concentrations during the first night-half expressed as the area under the curve (AUC) calculated according to the trapezoidal rule between 2320 hours and 0320 hours.

Sleep Analyses

Sleep architecture was determined according to standard polysomnographic criteria using EEG recordings from C3 and C4, diagonal EOG, and chin EMG (Rechtschaffen and Kales, 1968, details provided in the Supplementary Methods). For each night, total sleep time, ie, the time between the first detection of transition from sleep stage 1 to 2 and lights on, was used to calculate relative time spent in the different sleep

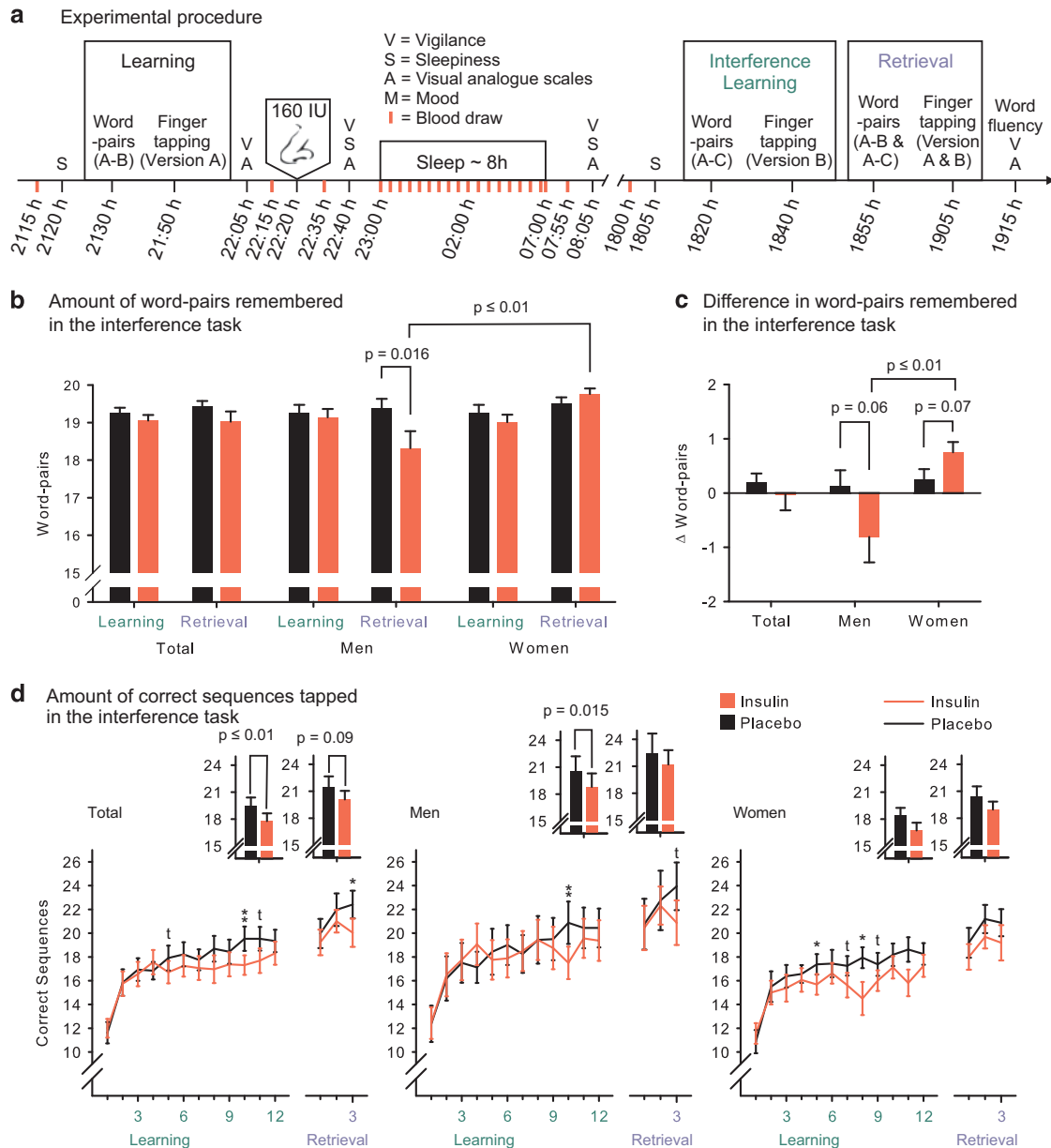


Figure 1 (a) Overview over the experimental procedure. (b) Mean (\pm SEM) absolute amounts of word-pairs remembered in the interference learning task in the evening after sleep, tested at learning and retrieval in the insulin (red) and placebo (black) conditions. (c) Mean (\pm SEM) differences between learning and retrieval performances of the interference word-pair task for insulin (red) and placebo (black). (d) Mean (\pm SEM) absolute amounts of correct sequences tapped in the interference learning task in the evening after sleep for each tapping block in the insulin (red) and placebo (black) conditions. Bar-plot inserts depict mean (\pm SEM) numbers of correct sequences during blocks 10–12 of learning and blocks 1–3 of retrieval. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.10$. $N = 32$ (16 men and 16 women).

stages, ie, wake, rapid eye movement (REM) sleep, and NonREM sleep stages 1, 2, 3, and 4.

Average power spectra were calculated at C3 and C4 for the first and the second 90 min of NonREM sleep. Normalized power spectra (ie, each frequency bin was normalized by the total power between 0.5 and 50 Hz) were calculated by Fast Fourier Transformation with a Hanning window applied to subsequent blocks of 2048 data points (~ 10.24 s, 3 blocks per 30 s epoch). The averaged spectra for each participant were filtered by a 5-point moving average to produce a smoothing of the FFT outcome. In the averaged spectra, mean power was determined for 0.5–1 Hz slow

oscillations, 1–4 Hz delta and the 12–15 Hz sleep spindle frequency bands for NonREM sleep and in the 4–8 Hz theta band for REM sleep.

Control Measures

Vigilance, sleepiness, mood, hunger, and thirst were repeatedly assessed with a reaction time task, the Stanford Sleepiness Scale, and visual analogue scales, respectively. General retrieval function was measured at retrieval using a word generation task (for details, see Supplementary Methods). At the end of each experimental session,

Table 1 Means, Standard Errors of the Mean (SEM), and *P*-values for *Post-hoc* Pairwise Comparisons for the Memory Tasks Learned before Sleep (ie, Amount of Correctly Recalled Words and Correctly Tapped Sequences); *N* = 32 (16 Men, 16 Women)

Measure	Insulin		Placebo		<i>P</i>
	Mean	SEM	Mean	SEM	
Word-pairs					
<i>Encoding</i>					
Total	19.19	(0.16)	19.31	(0.16)	0.59
Men	19.13	(0.22)	19.19	(0.23)	0.85
Women	19.25	(0.23)	19.44	(0.20)	0.58
<i>Retrieval</i>					
Total	13.78	(0.76)	13.94	(0.79)	0.83
Men	11.43	(0.99)	11.19	(1.05)	0.83
Women	16.13	(0.81)	16.69	(0.71)	0.55
<i>Difference</i>					
Total	-5.40	(0.75)	-5.38	(0.75)	0.96
Men	-7.69	(0.99)	-8.00	(0.95)	0.77
Women	-3.13	(0.83)	-2.75	(0.70)	0.69
Finger tapping					
<i>Encoding</i>					
Total	17.16	(0.83)	17.26	(0.96)	0.89
Men	18.25	(1.36)	18.81	(1.62)	0.62
Women	16.06	(0.93)	15.71	(0.94)	0.70
<i>Retrieval</i>					
Total	19.30	(1.13)	20.14	(1.17)	0.36
Men	20.37	(1.95)	21.02	(2.06)	0.65
Women	18.23	(1.12)	19.25	(1.16)	0.38
<i>Difference</i>					
Total	2.15	(0.68)	2.87	(0.61)	0.43
Men	2.12	(1.05)	2.21	(0.95)	0.95
Women	2.17	(0.90)	3.54	(0.76)	0.31

participants were asked whether they thought they had received insulin or placebo.

Statistical Analysis

For the analysis of sleep stages one female and one male participant were excluded because of partial data loss due to recorder malfunction. One male and three female participants were excluded from the EEG power analyses because of insufficient amounts of artifact-free data. For the analyses of blood parameters, two female participants were excluded because of insufficient data and one male participant was excluded from growth hormone analysis because 7 out of 20 values in the placebo condition were identified as outliers (ie, deviated from the mean by more than two standard deviations). Vigilance task data from four female participants could not be analyzed because of data loss. Exclusion of these participants did not alter the overall structure of the results. Statistical analyses generally relied on analyses of variance (SPSS version 21.0.0 for Windows) including the repeated

measures factors 'treatment' (insulin vs placebo) and 'time point' as appropriate, and the between-subjects factor sex (men vs women). Degrees of freedom were corrected according to the Greenhouse-Geisser procedure where appropriate. Pairwise comparisons were specified by *t*-tests unless stated otherwise. Pearson product-moment correlations were calculated to assess relationships between neuroendocrine, sleep-related, and behavioral parameters.

RESULTS

Memory Tasks

Whereas intranasal insulin administration did not affect the retrieval of declarative and procedural memory traces acquired before sleep, it impaired the capacity to encode interfering material in the evening following the experimental sleep night. Thus, intranasal insulin did not affect the number of recalled word-pairs learned before sleep (all $F_{(1,30)} \leq 1$, $p \geq 0.62$; see Table 1 for descriptive data and pairwise comparisons). In general, participants produced less word-pairs at retrieval than at encoding ($F_{(1,30)} = 111.45$ and $p \leq 0.001$) and women outperformed men ($F_{(1,30)} = 23.22$, $p \leq 0.001$), especially at retrieval ($F_{(1,30)} = 23.08$, $p \leq 0.001$). Women also performed better at the retrieval of the interfering word-pairs learned in the evening ($F_{(1,30)} = 6.03$ and $p = 0.02$; Figure 1b). Across all subjects, insulin treatment induced a trend-wise reduction in the amount of interfering word-pairs retrieved after sleep ($F_{(1,30)} = 3.07$, $p = 0.09$), which was driven by the men showing significantly impaired retrieval of the newly acquired interference word-pairs in the insulin condition ($F_{(1,30)} = 7.41$, $p = 0.011$ for treatment \times sex \times time point). Accordingly, men in contrast to women displayed a trend toward an insulin-induced impairment, whereas women showed signs of an improvement in performance occurring between encoding and retrieval of the interfering word-pairs (Figure 1c). There was a trend toward a positive relationship between the overnight retention of word-pairs encoded before sleep and the retention of interfering word-pairs for treatment-induced differences in the men ($r = 0.48$, $p = 0.06$).

In the finger sequence tapping task learned before sleep, participants improved across the retention interval in both conditions ($F_{(1,30)} = 29.59$ and $p \leq 0.001$), indicating that insulin did not affect its consolidation (all $F_{(1,30)} \leq 1$, $p \geq 0.43$; Table 1). However, insulin impaired performance on the interfering finger sequence tapping task learned in the subsequent evening ($F_{(1,30)} = 6.47$ and $p = 0.016$; Figure 1d), whereas all participants still improved from learning to retrieval ($F_{(1,30)} = 28.52$ and $p \leq 0.001$). This effect was independent of sex (all $F_{(1,30)} \leq 1.39$, $p \geq 0.25$). There was no effect of insulin on error rates in the finger sequence tapping task learned before sleep (all $F \leq 1.4$, $p \geq 0.23$) or after sleep (all $F \leq 2.74$, $p \geq 0.11$; see Supplementary Table 1).

Blood Parameters

Baseline values of all blood parameters determined at 2115 hours and 2215 hours did not differ between conditions (all $p > 0.14$). Intranasal insulin administration induced a sustained increase in growth hormone concentrations depending on point of time ($F_{(19,513)} = 4.66$, $p \leq 0.01$;

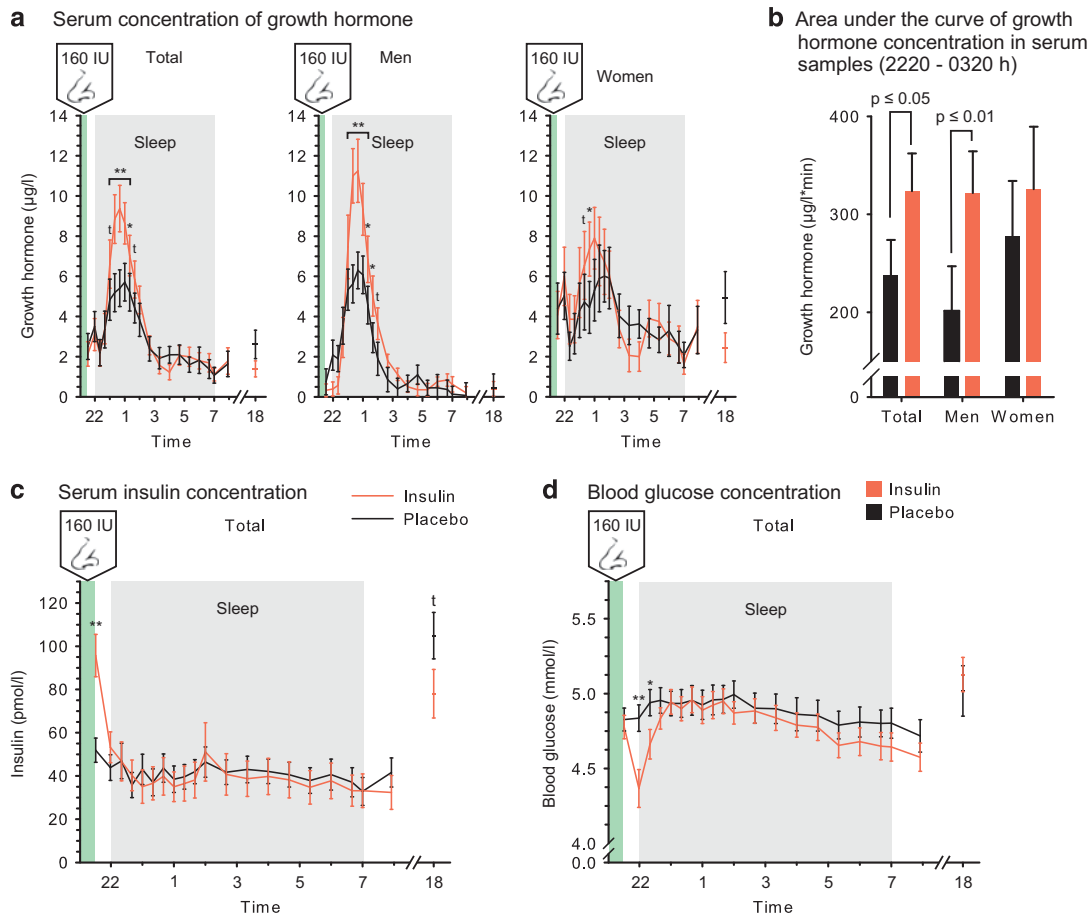


Figure 2 (a) Mean (\pm SEM) serum growth hormone concentrations following the intranasal administration of insulin (160 IU; red lines) and placebo (black lines). $N = 29$ (15 men and 14 women). Substance administration from 2220 to 2235 hours is indicated by the green bar. (b) AUC (\pm SEM) of serum growth hormone concentrations during the first half of the night (2320–0320 hours) in the insulin (red bars) and placebo (black bars) conditions. (c, d) Mean (\pm SEM) serum insulin and blood glucose concentrations in the insulin (red lines) and placebo (black lines) conditions. $N = 30$ (16 men and 14 women). All data were individually baseline adjusted with reference to measurements at 2115 and 2215 hours. ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

Figure 2a). Accordingly, growth hormone AUC values for the first night-half (2320–0320 h; Figure 2b) were increased by insulin ($F_{(1,27)} = 5.14$, $p = 0.03$). Sex did not significantly modulate this effect ($F \leq 1$, $p = 0.34$ for treatment \times sex; men, $t_{(14)} = 2.90$, $p = 0.012$; women, $t \leq 1$, $p = 0.46$).

Serum insulin levels in general slightly decreased throughout the night ($F_{(19,532)} = 12.13$, $p \leq 0.001$; Figure 2c and Supplementary Figure 1A). Intranasal insulin in comparison to placebo administration induced a transient increase in insulin concentrations immediately after substance administration at 2235 hours ($t_{(29)} = 4.32$, $p \leq 0.001$; $F_{(19,532)} = 5.27$, $p \leq 0.001$ for treatment \times time point), whereas insulin levels were closely comparable between conditions during the rest of the night. At retrieval testing on the subsequent afternoon, insulin levels tended to be lower in the insulin compared with the placebo condition ($t_{(29)} = -1.84$, $p = 0.08$). Blood glucose concentrations decreased until dawn ($F_{(19,532)} = 9.50$, $p \leq 0.001$; Figure 2d and Supplementary Figure 1B) and showed a transient decrease after intranasal insulin compared with placebo administration (2300 hours: $t_{(29)} = -2.96$, $p \leq 0.01$; 2320 hours: $t_{(29)} = -2.14$, $p = 0.04$; $F_{(19,532)} = 2.78$, $p = 0.012$ for treatment \times time point). Signs of decreased blood glucose levels in the insulin compared with the placebo condition in the male participants

(Supplementary Figure 1B) were not confirmed by respective overall analyses ($p > 0.42$), which neither indicated systematic treatment-related differences between sexes ($p > 0.17$).

Correlational analyses covering the whole sample of participants revealed that the treatment-induced increases observed for growth hormone during the first night-half (AUC_{growth hormone} 2320–0320 hours) and for serum insulin immediately after substance administration (2235 hours) were inversely related ($r = -0.43$, $p \leq 0.05$). Respective correlations between the growth hormone AUC and the treatment-induced drop in blood glucose levels (mean level at 2300 hours and 2320 hours) did not reach significance ($r = -0.32$, $p = 0.09$).

Sleep

Intranasal insulin administration did not influence polysomnographically determined sleep stages in the whole sample of subjects (all $t \leq 1$, $p \geq 0.71$; Table 2). The female participants showed a trend toward less REM sleep in the insulin compared with the placebo condition ($t_{(14)} = -1.82$, $p = 0.096$; see Supplementary Tables 2 and 3 for results in men and women, respectively). Control measures indicated that subjective sleepiness assessed in the afternoon after the

Table 2 Means, Standard Errors of the Mean (SEM), and *P*-values for Pairwise Comparisons between Conditions Regarding Time Spent in Different Sleep Stages (Relative to Total Sleep Time; *N* = 15 Men and 15 Women), EEG Power (Percent of Total Power between 0.5 and 50 Hz; *N* = 15 Men and 13 Women) in the Slow Oscillation (0.5–1 Hz) and Sleep Spindle (12–15 Hz) Band during the First and the Second 90 min of NonREM Sleep, Reaction Speed (1/Reaction Time; *N* = 16 Men and 12 Women), and Sleepiness (According to the Stanford Sleepiness Scale; *N* = 16 Men and 16 Women)

Measure	Insulin		Placebo		<i>P</i>
	Mean	SEM	Mean	SEM	
<i>Sleep stage</i>					
Wake (%)	1.96	(0.6)	1.72	(0.53)	0.76
S1 (%)	7.76	(0.91)	7.54	(0.93)	0.76
S2 (%)	54.09	(1.37)	54.50	(1.46)	0.74
S3 (%)	8.01	(0.72)	8.08	(0.76)	0.87
S4 (%)	7.57	(1.02)	7.51	(1.08)	0.93
REM (%)	19.68	(0.89)	19.58	(0.92)	0.93
Total sleep (min)	460.3	(3.33)	458.73	(4.93)	0.71
<i>Slow oscillation band (0.5–1 Hz)</i>					
C3 1st NonREM	33.8	(1.05)	33.24	(0.88)	0.57
C4 1st NonREM	33.83	(0.89)	34.46	(0.78)	0.28
C3 2nd NonREM	35.46	(0.95)	37.46	(1.52)	0.23
C4 2nd NonREM	36.12	(1.03)	36.82	(0.95)	0.42
<i>Sleep spindle band (12–15 Hz)</i>					
C3 1st NonREM	2.82	(0.25)	2.91	(0.24)	0.64
C4 1st NonREM	2.81	(0.27)	2.77	(0.24)	0.79
C3 2nd NonREM	4.06	(0.37)	4.04	(0.40)	0.95
C4 2nd NonREM	4.03	(0.38)	3.92	(0.40)	0.61
<i>Reaction speed</i>					
2205 hours	2.93	(0.06)	3.01	(0.06)	0.06
2240 hours	2.92	(0.06)	2.85	(0.07)	0.07
0805 hours	3.04	(0.07)	2.97	(0.07)	0.17
1915 hours	2.96	(0.07)	2.99	(0.07)	0.56
<i>Sleepiness</i>					
2120 hours	2.84	(0.17)	3.00	(0.20)	0.33
2240 hours	3.44	(0.17)	3.50	(0.20)	0.74
0805 hours	2.81	(0.18)	2.97	(0.21)	0.36
1805 hours	2.13	(0.19)	2.47	(0.15)	0.09

experimental night was decreased in the insulin condition (see Supplementary Results).

A more fine-grained polysomnographic analysis of EEG power between 0.1 and 35 Hz revealed that intranasal insulin increased the amount of delta power in the second 90 min cycle of NonREM sleep in males. Thus, insulin induced a significant increase in EEG power in the delta range (1–4 Hz) at both electrode locations (C3 and C4) during the second 90 min of NonREM sleep, and a reduction in the spindle band (12–15 Hz) at C3 during the first 90 min of NonREM

sleep (see Figure 3 for the total sample and Supplementary Figures 2 and 3 for men and women, respectively). The insulin-induced increase in delta band power during the second 90 min of NonREM sleep was driven by an effect in the male participants (C3: $t_{(14)} = 3.08$, $p \leq 0.01$; C4: $t_{(14)} = 3.24$, $p \leq 0.01$; Figure 3d). Accordingly, there was a significant interaction between the factors treatment, sex, and time point ($F_{(1,25)} = 4.36$, $p \leq 0.05$). There was no significant relation between the insulin-induced increase in growth hormone concentrations during the first night-half and the increase in delta power during the second NonREM cycle ($p > 0.38$). There was also no effect of insulin on theta power during REM sleep (all $p > 0.20$). See Table 3 for a summary of the observed effects.

DISCUSSION

The present study aimed at investigating whether central nervous insulin signaling interacts with the beneficial effect of sleep on memory consolidation by administering intranasal insulin to healthy subjects between learning declarative and procedural memory contents and a retention interval containing 8 h of nocturnal sleep. Insulin treatment increased growth hormone concentrations during the first night-half and relative EEG delta band power during the second NonREM sleep cycle. Insulin did not directly affect measures of sleep-dependent declarative or procedural memory consolidation but impaired the delayed acquisition of new declarative and procedural memory contents that interfered with the memory traces learned before sleep. These findings suggest that the improving effect of intranasal insulin administration on memory function observed in previous studies (Benedict *et al*, 2004, 2007; Craft *et al*, 2012; Reger *et al*, 2008b) is not primarily conveyed by sleep-dependent mechanisms, although central nervous insulin delivery impacts neuroendocrine and neurophysiological sleep patterns.

The predominance in men rather than women of insulin's effects on sleep-associated neurophysiology and subsequent cognitive function ties in with previous observations of sex differences in the response to intranasal insulin in the metabolic and cognitive domain (Benedict *et al*, 2008; Hallschmid *et al*, 2004; Krug *et al*, 2010). Notably, immediate improving effects of intranasal insulin on declarative memory were found in healthy women (on estrogen-dominant oral contraceptives), whereas men did not benefit (Benedict *et al*, 2008). Fittingly, in the insulin condition of the present study, female participants showed a trend toward enhanced memory for interfering declarative contents, whereas men rather displayed deterioration. Interestingly, sex differences and an influence of the menstrual cycle have also been found for the general effect of sleep on memory function (Genzel *et al*, 2012). As all female participants of the present study were taking contraceptives, additional studies in free cycling women are needed to obtain further insight into this pattern. Here, the inclusion of wake control groups, which was not done in the present study, would further substantiate the critical involvement of sleep-dependent processes. Moreover, it remains to be seen whether long-term intranasal insulin administration impacts sleep-related memory formation.

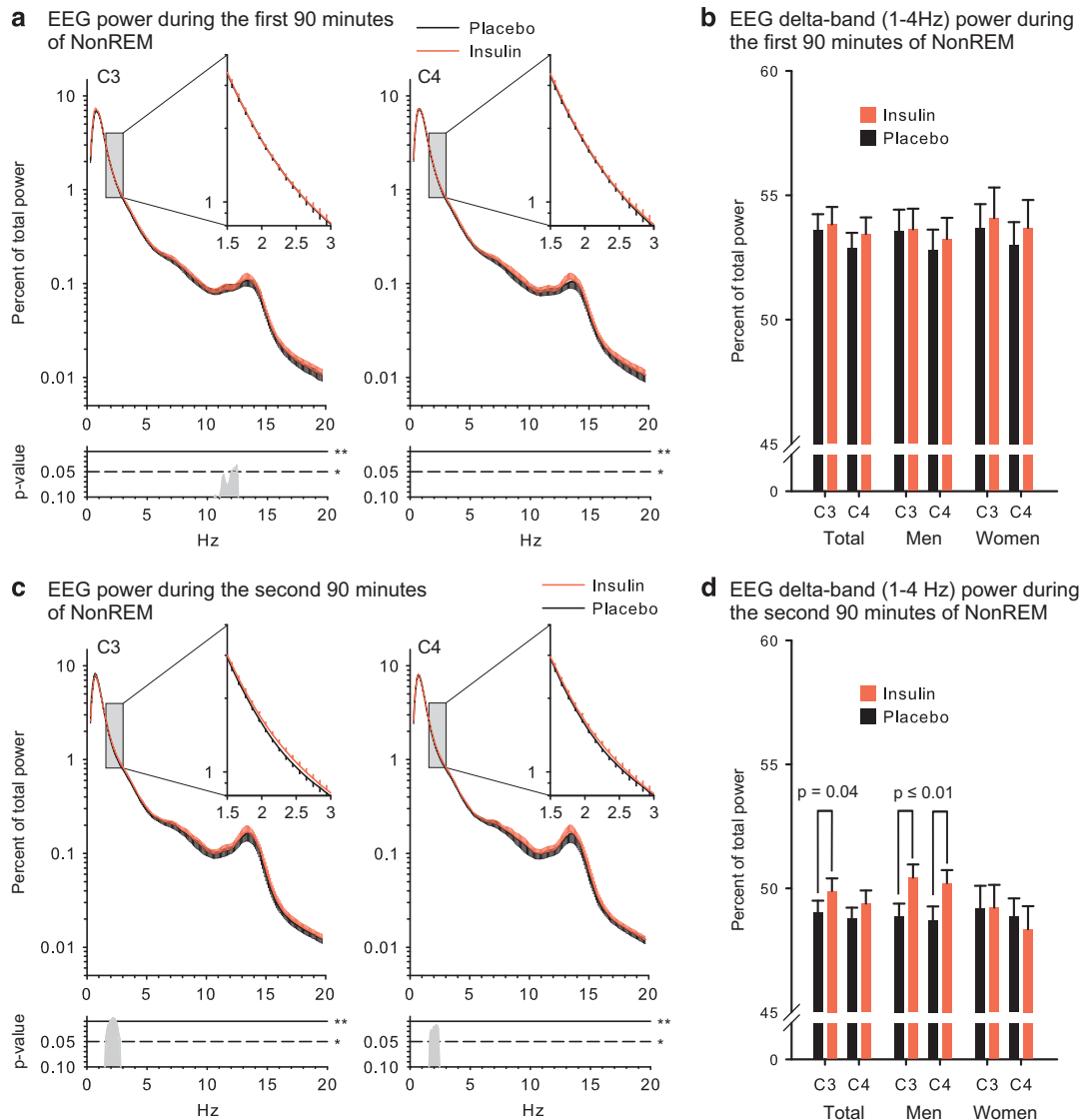


Figure 3 EEG power during NonREM sleep. (a, c) Mean (\pm SEM) normalized EEG power between 0.5 and 20 Hz for electrode positions C3 (left) and C4 (right) in the insulin (red) and placebo (black) conditions during (a) the first 90 min and (c) the second 90 min of NonREM sleep; respective inserts show EEG power between 1.5 and 3 Hz. Below each panel, p -values for pairwise comparisons between conditions for each frequency bin (0.01 Hz) are indicated. (b, d) Mean (\pm SEM) cumulative EEG power in the delta band (1–4 Hz) at C3 and C4 electrode positions in the insulin (red) and placebo (black) condition during (b) the first 90 min and (d) the second 90 min of NonREM sleep recorded in the total sample of subjects as well as the men and women. $N = 28$ (15 men and 13 women). $**P \leq 0.01$, $*P \leq 0.05$.

Beneficial effects of insulin on memory function can be attributed to insulin receptors located in brain regions relevant for memory formation, such as the hippocampus and connected limbic brain structures (Unger *et al*, 1991). Insulin can induce AMPA receptor internalization, which leads to long-term depression (Man *et al*, 2000). Also, insulin phosphorylates AMPA receptors and leads to the overexpression of PKM ζ (Adzovic and Domenici, 2014), and downregulating hippocampal insulin receptor function impairs long-term potentiation and spatial memory (Grillo *et al*, 2015). Since both long-term depression and long-term potentiation contribute to the establishment of memory traces in the hippocampus (Goh and Manahan-Vaughan, 2015; Born and Feld, 2012), insulin may exert some of its memory-improving effects by modulating these plastic processes. The strong effect of estrogen on synaptic plasticity

(Baudry *et al*, 2012) may also interact with respective insulin effects and yield the sex differences found here and in other studies (Benedict *et al*, 2008; Krug *et al*, 2010). Insulin also potentiates NMDA receptor activity (Liu *et al*, 1995) via delivery of NMDA receptors to the cell surface (Skeberdis *et al*, 2001) and NMDA receptor phosphorylation (Christie *et al*, 1999). Shifts in NMDA receptor activity like this may induce long-lasting meta-plastic changes (Hulme *et al*, 2013). Of note, NMDA and AMPA receptors are essential for the sleep-dependent consolidation of cortical-procedural tasks (Gais *et al*, 2008) and NMDA receptors are involved in the sleep-dependent consolidation of declarative memories (Feld *et al*, 2013a). Our finding that the retrieval of memory contents is not altered by central nervous insulin administration before a retention interval containing around 7.5 h of sleep—which

Table 3 Overview Over Relevant Variables, Underlying Theoretical Assumptions, and Respective Insulin Effects

Variable	Concept	Insulin effect		
		Men	Women	All
Word-pair retention over sleep	Sleep-associated declarative memory consolidation	↔	↔	↔
Finger sequence tapping retention over sleep	Sleep-associated procedural memory consolidation	↔	↔	↔
Word-pair learning after sleep (retrieval minus encoding)	Sleep-associated re-establishment of learning capacity	(↓)	(↑)	(↓)
Finger sequence tapping learning after sleep	Sleep-associated re-establishment of learning capacity	↓	↓	↓
Growth hormone secretion during early sleep	Slow wave sleep-dependent increase in secretion	↑	↑	↑
EEG delta power (second 90 min of NonREM sleep)	Homeostatic reduction in delta power across sleep	↑	↔	↔

↔: No effect, ↑: enhancing/increasing effect, ↓: impairing/reducing effect; statistical trends are indicated by bracketed arrows. Note that differential effects in both sexes are only indicated when respective treatment × sex terms are significant.

was obtained in a sample of sufficient size to detect also more subtle effects—suggests that insulin does not directly act on sleep-related neurochemical changes with relevance for memory formation. Alternatively, the peptide might improve memory function by enhancing regional glucose turnover (Osborne *et al*, 2015). Thus, four months of daily intranasal insulin delivery to adults with mild cognitive impairment or mild Alzheimer's disease not only improved memory function but also prevented the decrease in brain glucose uptake observed in placebo-treated participants (Craft *et al*, 2012). Similar mechanisms might be behind acute (and therefore sleep-unrelated) enhancements of memory function in healthy (Benedict *et al*, 2008) and cognitively impaired subjects (Reger *et al*, 2008a) and also contribute to effects of long-term treatments (Benedict *et al*, 2004, 2007; Reger *et al*, 2008b).

The intranasal insulin-induced increase in growth hormone concentrations is a new and interesting finding. In accordance with previous experiments (Benedict *et al*, 2011; Krug *et al*, 2010), intranasal insulin delivery was associated with a transient spillover of insulin into the circulation and a respective drop in blood glucose levels, which, however, clearly remained above the hypoglycemic threshold of 3.6–3.8 mmol/l where hormonal counter regulatory responses including an increase in growth hormone release are elicited (Cryer, 1997). The decrease in blood glucose did not correlate with the rise in growth hormone levels, which, moreover, was inversely rather than positively related to the increase in circulating insulin, an observation in line with animal studies indicating that insulin suppresses growth hormone release at the level of the pituitary (Gahete *et al*, 2013). Thus, the promotion of somatotrophic activity by intranasal insulin administration to the brain most likely can be attributed to central nervous mechanisms, which to our knowledge have not been investigated so far. Interestingly, the strong increase in growth hormone concentrations—as well as central nervous insulin delivery per se—remained without immediate effect on sleep-dependent memory consolidation. Fittingly, the infusion of somatostatin during sleep effectively blocks growth hormone and insulin release in healthy humans but does not affect memory formation (Gais *et al*, 2006), although somatotrophic activity has been repeatedly indicated

to be positively related to memory function (Vitiello *et al*, 2006; Hallschmid *et al*, 2011).

As growth hormone release is strongly related to NonREM sleep (Van Cauter *et al*, 1998), it is tempting to speculate that the insulin-induced increase in growth hormone concentrations might have triggered the subsequent enhancement in EEG delta band power. However, the two effects were statistically unrelated, and systemic application of growth hormone or growth hormone-releasing hormone does not affect human sleep (Kern *et al*, 1993), which renders such a connection improbable. Nocturnal insulin secretion is entrained to NonREM sleep phases (Kern *et al*, 1996), and peripheral and intracerebroventricular administration of insulin to healthy and diabetic rats increases the time spent in NonREM sleep (Danguir and Nicolaidis, 1984; Sangiah *et al*, 1982). Although relative time spent in different sleep stages was not affected by intranasal insulin in the present study, our finding of an insulin-induced increase in delta band power during the second 90 min of NonREM sleep in the men indicates that insulin intensifies NonREM sleep also in humans. Specifically, the occurrence of this increase in the second 90 min of NonREM sleep may be the result of central nervous insulin interfering with the homeostatic reduction of sleep pressure that normally manifests in waning delta band activity during the night (Borbely and Achermann, 1999). The absence of a respective effect in women may be related to estrogen that is known to alter sleep architecture and homeostatic regulation in rats (Deurveilher *et al*, 2011). Insulin has been found to be expressed in GABAergic neurogliaform cells in rodent cerebral cortex (Molnar *et al*, 2014) and can lead to rapid insertion of post-synaptic GABA_A receptors (Wan *et al*, 1997). Tonic GABA_A-receptor activation strongly increases the amount of NonREM sleep and delta power (Lancel, 1999). Assuming that insulin enhanced delta activity via respective mechanisms in the present study, the absence of a directly measurable effect on memory consolidation would be in line with our previous observation that inhibiting GABA re-uptake promotes slow wave activity without benefiting memory consolidation (Feld *et al*, 2013b).

Our finding that intranasal insulin administration before sleep compromises the learning of interfering memory contents in the subsequent evening most likely was not due to a direct effect of exogenous insulin at the retrieval session

because the peptide can be assumed to have cleared the system during the preceding 20-h delay (Born *et al*, 2002). It might be argued that the impairment in new learning was a consequence of improved overnight consolidation interfering with encoding capacity. However, retrieval of the originally encoded word-pairs was comparable between conditions. Moreover, insulin-associated differences in the overnight retention of word-pairs were positively rather than negatively associated with respective differences in the performance on the interfering words. The enhanced brain insulin signal might rather have interacted with a supposed neurophysiological key feature of sleep, ie, its beneficial effect on subsequent new learning (Feld and Diekelmann, 2015), which is thought to be achieved by the renormalization of synaptic weights during NonREM's slow wave sleep (Tononi and Cirelli, 2014) and REM sleep theta activity (Born and Feld, 2012). According to this theory, the encoding of new information during wakefulness is mainly achieved by widespread potentiation, which must be subsequently compensated to sustain the brain's ability to incorporate new information. The concept that sleep, and in particular NonREM sleep, fosters the ability to learn new information has received ample empirical support (eg, Antonenko *et al*, 2013; Yoo *et al*, 2007). Moreover, REM sleep has been shown to renormalize firing rates in the hippocampus (Grosmark *et al*, 2012). However, in the present experiments, delta activity was increased and REM sleep theta power remained unchanged after insulin administration. Therefore, it seems more plausible that the plasticity-mediating effects of central nervous insulin outlined above exert a direct detrimental effect on the capacity to learn new contents (eg, by inducing unselective potentiation in hippocampal synapses) even when subjective tiredness is relatively reduced, as observed in the insulin condition of our study. It is well conceivable that insulin disturbs the machinery of an active decay (eg, PKM ζ) that is constantly erasing irrelevant memories (Hardt *et al*, 2013), although clearly more work is necessary to delineate insulin's specific effects on (sleep-related) changes in synaptic plasticity.

In sum, our study indicates that in healthy humans intranasal insulin enhances the sleep-associated rise in growth hormone concentrations but that insulin's beneficial effect on memory function is not primarily due to acute improvements in sleep-dependent memory consolidation. This finding highlights that plastic processes during sleep can differ from the wake state, as it contrasts the acutely improving effect of intranasal insulin on memory encoding (Benedict *et al*, 2008; Krug *et al*, 2010). Still, central nervous insulin appears to act on sleep-associated mechanisms that diminish the subsequent encoding of interfering information, suggesting an involvement of changes in meta-plastic or homeostatic processes. Thus, insulin might be assumed to benefit memory formation by reducing the interfering influence of new information, eg, by perturbing processes of active forgetting during sleep. These results shed new light on the potential of intranasal insulin as a memory-aiding drug, which is the subject of an increasing number of research efforts (Spetter and Hallschmid, 2015).

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