The Significance of Sigma Neurofeedback Training on Sleep Spindles and Aspects of Declarative Memory

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The functional significance of sleep spindles for overnight memory consolidation and general learning aptitude as well as the effect of four 10-minute sessions of spindle frequency (11.6–16 Hz, sigma) neurofeedback-training on subsequent sleep spindle activity and overnight performance change was investigated. Before sleep, subjects were trained on a paired-associate word list task after having received either neurofeedback training (NFT) or pseudofeedback training (PFT).

Although NFT had no significant impact on subsequent spindle activity and behavioral outcomes, there was a trend for enhanced sigma band-power during NREM (stage 2 to 4) sleep after NFT as compared to PFT. Furthermore, a significant positive correlation between spindle activity during slow wave sleep (in the first night half) and overall memory performance was revealed. The results support the view that the considerable interindividual variance in sleep spindle activity can at least be partly explained by differences in the ability to acquire new declarative information.

We conclude that the short NFT before sleep was not sufficient to efficiently enhance phasic spindle activity and/or to influence memory processing. NFT was, however, successful in increasing sigma power, presumably because sigma NFT effects become more easily evident in actually trained frequency bands than in associated phasic spindle activity.

INTRODUCTION

The Significance of Sleep Spindles for Memory Processes

By now, the involvement of rapid-eye-movement (REM) sleep and slow-wave sleep (SWS) in memory consolidation processes has gained substantial support by a large body of research (for review see Maquet, Nader, & Smith, 2003). In contrast, the functional significance of stage 2 sleep in this matter remains relatively unexplored. In the investigation of sleep-related learning and memory processes, researchers have only recently begun to

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97

also consider stage 2 non-REM (NREM) sleep and its characteristic features—the sleep spindles—as possible candidates for "offline" information processing. Human stage 2 (S2) sleep was at first considered to play a role in the processing of various types of motor memories (Smith & MacNeill, 1994; see Nader & Smith, 2003 for review) and was hypothesized to be of particular importance for optimal performance on simple motor procedural memory tasks (Smith & MacNeill, 1994; Meier-Koll, Bussmann, Schmidt, & Neuschwander, 1999; Tweed, Aubrey, Nader, & Smith, 1999). Reports followed which strengthened the finding that the thalamocortically generated sleep spindles are indeed related to working memory (Schiffelholz & Aldenhoff, 2002), verbal memory (Brière, Forest, Lussier, & Godbout, 2000), and especially motor procedural learning (Fogel, Jacob, & Smith, 2001; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). Interestingly, Gais, Mölle, Helms, and Born (2002) found that (stage 2) sleep spindles are also enhanced after *declarative learning* as compared to a non-learning control task of similar cognitive strain. Recently, evidence accumulated which indicated a positive relationship between sleep spindles and overnight consolidation of declarative memories (Schabus et al., 2004; Clemens, Fabo, & Halasz, 2005). More specifically, Schabus et al. (2004) demonstrated that only those subjects who were able to enhance their spindle activity following a learning as compared to a non-learning control task also showed overnight improvement in declarative memory (i.e., word-pair association) performance.

Additionally, sleep spindles have been hypothesized to be associated with "general learning aptitude" or an individual's general ability to acquire new information (for review see Nader & Smith, 2003). Especially, sleep spindle measures (of a normal night's sleep) have been positively related to non-verbal intelligence scores (Patterson, Gluck, & Mack, 1983; Fogel et al., 2001; Nader & Smith, 2001; Bódizs et al., 2005; Schabus et al., 2006), explicit (Gais et al., 2002), and implicit learning abilities (Brière et al., 2000).

Decreases in sleep spindle density or duration, on the other hand, have been reported in conditions of cognitive decline in children (Shibagaki, Kiyono, & Watanabe, 1980; Quadens, 2003) and in age related diseases such as Alzheimer's syndrome (Montplaisir, Petit, Lorrain, Gauthier, & Nielsen, 1995). Consequently, it was speculated that the considerable inter-individual variance in sleep spindle density (Silverstein & Levy, 1976; Werth, Achermann, Dijk, & Borbély, 1997; De Gennaro, Ferrara, Vecchio, Curcio, & Bertini, 2005) might be partly related to differences in learning capabilities and mental functioning.

Taken together, studies investigating possible relationships between sleep spindle activity and memory performance have provided experimental evidence for an involvement of spindle oscillations in different types of memory and for both overnight memory consolidation and general learning ability.

Neurofeedback

Neurofeedback (NF) refers to an operant conditioning paradigm where participants can learn voluntary control of distinct parameters of their electrical brain activity as measured by the electroencephalogram (EEG). Learned self-regulation of specific EEG frequency components has proven to be of considerable value in clinical settings with applications in various pathologies such as epilepsy (Rockstroh et al., 1993), ADHD (Vernon, Frick, & Gruzelier, 2004) and schizophrenia (Gruzelier, Hardmann, Wild, & Zaman, 1999). Additionally, more recent research focused on healthy individuals providing evidence that

subjects are able to gain some control over different EEG components and thereby increase performance levels. These reports pointed out that distinct NF-training (NFT) protocols can be successfully used to improve attentional processing (Egner & Gruzelier, 2004), to increase accuracy in working memory tasks (Vernon et al., 2003) or to improve performance in mental rotation tasks (Hanslmayr, Sauseng, Doppelmayr, Schabus, & Klimesch, 2005). Taken together, the fascinating idea emerged that NF provides a tool to stimulate and/or learn how to regulate cerebral activity which consequently may lead to changes in cognitive processing. Based on the previously reported associations between sleep spindle activity and memory performance the present study was designed to investigate (among others) the possibility that training healthy individuals with sigma power NFT (i.e., in the sleep spindle frequency range) might result in enhanced spindle production during subsequent sleep, less wake time after sleep onset and in the best case even to enhanced memory performance. The idea is inspired by findings from Barry Sterman (1970) demonstrating that 12–14 Hz conditioning of the sensorimotor rhythm (SMR) over a period of 2–4 weeks (in cats) can successfully induce a facilitation of spindle burst sleep and decrease sleep fragmentation (i.e., prolong sleep epochs). Moreover, SMR neurofeedback training (applied several times per week) has previously been effectively used in treating patients suffering from psychophysiologic insomnia (Hauri, 1981). The amount of feedback learning correlated significantly with sleep improvement and was interpreted to be based on a direct influence of NFT on spindling mechanisms.

In the following, the rather multifaceted research questions of the present study are summarized. At first, we will explore the effectiveness of NFT for influencing sleep parameters (cf. 3.1). Secondly, we address the immediate effects of NFT on (i) EEG-spectra, (ii) sleep spindle activity, and (iii) sigma band power and (iv) try to explore whether sigma NFT before sleep is effective in influencing aspects of declarative memory performance (cf. 3.2). As a last and third point, we address the more general relationship of sleep spindles and (i) declarative overnight memory performance as well as the relation of spindles with (ii) an individual's general ability to acquire new declarative information (cf. 3.4).

METHOD

Subjects

Subjects were 11 right-handed high school and university students aged between 18 and 26 ($M = 20.8$ years, $SD = 2.8$ years) who had prior to the present experiment participated in a comprehensive neurofeedbacktraining (NFT) study. From the pool of 60 subjects of that NFT-study only those subjects were asked to participate who previously had been able to enhance their alpha band-power (so called "NFT responders"). NFT in the prior NFT-experiment was considered successful when subjects were able to continuously increase their alpha power from the first to the last (6th) training session. Out of 19 responders, 11 subjects (four males and seven females) agreed to participate in the study. Subjects had no history of severe organic or mental illness, no sleep disturbances (Pittsburgh Sleep Quality Index Global Score [PSQI-TS ≤ 5]; Buysse, Reynolds, Monk, Bermn, & Kupfer, 1989) and no signs of mood disorders (Self-Rated Anxiety Scale [SAS raw score < 36]; Zung, 1971; Self-Rated Depression Scale [SDS raw score < 40]; Zung, 1965). Furthermore,

no strong morning or evening types were part of the study sample, as revealed by the Morningness–Eveningness Questionnaire (Horne & Oestberg, 1976). For the 2 week study period subjects reported sleep habits and sleep quality by daily sleep diaries. Subjects were required to maintain a regular sleep-wake cycle across the study period which was monitored by wrist-worn actigraphs. Prior to participation all subjects were fully informed about all study procedures and gave written informed consent. Subjects received a compensation of €70.- for taking part in the study.

Procedure

Subjects had to attend the laboratory three times separated by 7 (± 1) days (cf. Fig. 1). The first visit was carried out one week before the first experimental night in the sleep laboratory and served as baseline assessment with documentation of sleep habits, sleep quality (PSQI), anxiety (SAS), depression (SDS) and chronotype (D-MEQ). Subjects were equipped with a wrist-worn actigraph and handed out sleep logs for the following week. One week later, subjects reported to the sleep laboratory at 19:30 for the first experimental night, which was separated from the second experimental night by another week. Both experimental sessions served as learning conditions with subjects performing a declarative memory task before going to sleep. The first and second experimental night differed only in the type of neurofeedback training performed before the declarative learning task in the evening (approximately three hours before the beginning of sleep). Subjects received either neurofeedback training (NFT) or pseudo feedback training (PFT), with the order of NFT and PFT being counterbalanced across subjects. All-night polysomnographic recordings (PSG) started between 23:00 and 00:00 and were terminated after the subject's habitual total sleep time or after eight hours of sleep. Immediately before and after sleep all subjects rated mood, affectivity, drive and tiredness on a 100 mm visual analogue scale.

Neurofeedback protocol

Before the beginning of NFT, two reference EEG conditions were recorded for two minutes each, one with eyes closed (ref$_{EC}$) and another one with eyes open (ref$_{EO}$) while subjects viewed pictures of animals or landscape scenes on a 15″ computer screen. NFT was carried out in four consecutive training sessions of ten minutes each, separated from another by two-minute breaks. In the NFT condition subjects were instructed to try to increase the clarity of pictures presented on a monitor in front of them. Every time subjects reached the individually defined NFT criteria (increase in 11.6–16 Hz sigma power at electrode position Cz by 2–4 standard deviations and EMG and EOG activity below individual threshold) a short acoustic tone was presented and the clarity of the picture increased by 1 of 7 increments. The adjustment of 2 to 4 standard deviations was done in order to keep the number of feedbacks more or less constant for all subjects. The PFT session only differed with respect to the visual and auditory feedback that was given randomly from an inactive EEG channel. The number of feedbacks received was held similar during the two experimental conditions by individually adjusting (pseudo-)feedback parameters accordingly.
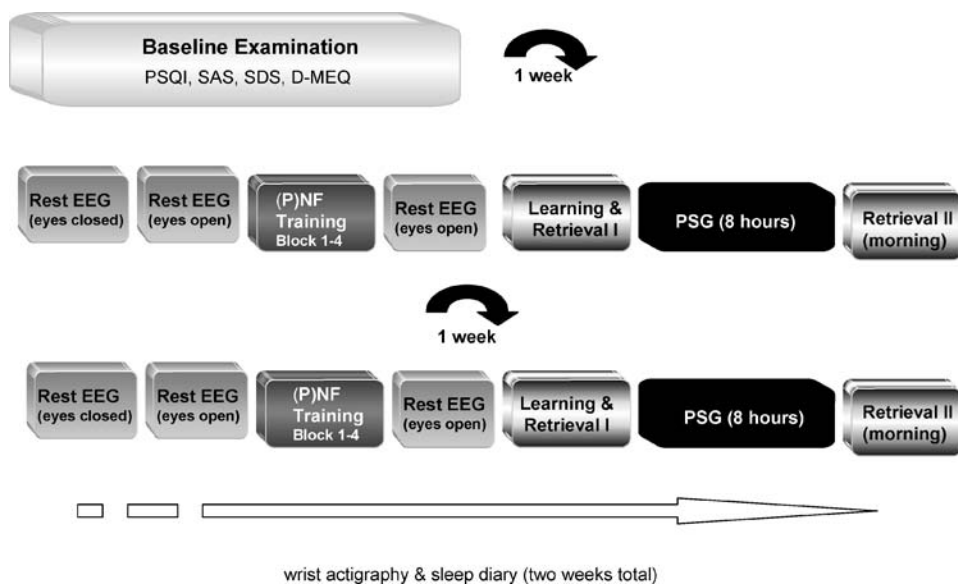
Fig. 1. Shown is the study design over a period of 2 weeks. Eleven subjects came to the sleep laboratory at three occasions to undergo an entrance examination and to accomplish twice a declarative memory task after being trained by either sigma (11.6–16 Hz) neurofeedback (NF) or pseudofeedback (PNF). Afterwards, subjects spent the night in the laboratory with full polysomnographic montage. In the morning after awakening individuals were again tested for memory performance. PSQI, Pittsburgh Sleep Quality Index; SAS, Self-Rated Anxiety Scale; SDS, Self-Rated Depression Scale; PSG, polysomnography.

Memory testing

After NFT/PFT, subjects had to complete a paired-associate word list task (from Plihal and Born, 1997), however, in an adapted and newly randomized version consisting of 80 randomly related word-pairs. During encoding, each word-pair was presented in white on black background on a 15″ computer screen for 1500 ms, followed by a 5000 ms period with only a white fixation cross visible. During this period, subjects were instructed to encode the word-pair by imagining a visual relation between the two words (e.g., a *snowman* with a *carrot* as nose). Then, the fixation cross skipped from white to grey giving the subject the signal to stop encoding, relax and wait for the next word-pair to be presented. The grey fixation cross was visible for 3500 ms. Altogether, the encoding session lasted thirteen minutes. Recall performance was tested twice by a cued-recall test—presenting only the first words of the previously learned word-pairs—, once in the evening approximately 10–15 min after learning and once in the morning approximately half an hour after the end of sleep. The word and a question mark were visible on the screen for a maximum period of 6500 ms. Whenever subjects remembered the corresponding word, they had to first press a button with their right thumb and then verbally report it (e.g. *carrot* in response to *snowman*). If subjects did not press the button within the 6500 ms period, the presentation automatically skipped to the next word. Two parallel versions of the paired-associate word list task consisting of different word-pairs were used on the two experimental days in order to circumvent training effects.

The recall score consisted of (a) the number of identically correct responses and (b) the number of semantically correct responses. Correct and unambiguously semantically correct words were counted (e.g., *desk* instead of *table*) and weighted by 1 and 0.5, respectively. The so obtained response score values were converted into percentages [memory performance$_\%$ = response score/80 × 100] and are subsequently referred to as "correctly recalled."

EEG Recordings

EEG signals were recorded using Synamps EEG amplifiers (NeuroScan Inc.). Signals were digitized online with 250 Hz sampling rate and filtered using a 0.10 Hz high-pass filter and a 70 Hz low-pass filter. Seven gold-plated silver electrodes (Fz, C3, Cz, C4, Pz, O1, O2) were attached according to the international 10/20 system (Jasper, 1958). Signals were online referenced to Fcz. In addition to the seven EEG channels, five electro-oculogram (EOG) channels, and a bipolar electromyogram (EMG) channel on the chin, one bipolar electrocardiogram (ECG) and one respiratory channel (chest belt measuring chest wall movements) were recorded.

EEG was recorded throughout the whole experiment with visual artifact rejection being done offline on the EEG of the experimental conditions. Night recordings were checked for major artifacts like movements and muscle activity. Baseline/reference conditions were recorded before and after NFT/PFT. Power estimates were derived by spectral analysis of 2 second epochs (frequency resolution of 0.5 Hz) and for seven frequency bands (delta [0–3.9 Hz] theta [3.9–5.9 Hz], L-1 alpha [5.9–7.8 Hz], L-2 alpha [7.8–9.8 Hz], U-alpha [9.8–12 Hz] and sigma [11.5–15.9 Hz]).

Sleep Staging

Sleep staging was performed manually according to standard criteria (Rechtschaffen & Kales, 1968) by analyzing 30-sec sleep epochs using Sleep Explorer 1.0.0 software. The amount of time spent in each sleep stage is expressed as percentage of sleep period time (SPT, time from first sleep onset to last awakening in the morning). The full nights of sleep (approx. eight hours) were additionally divided into quartiles in order to allow for a more detailed analysis of sleep spindles and sleep architecture over the course of the night.

Sleep Spindle Detection

The detection of sleep spindles was based on an automatic sleep-spindle detection procedure developed by Schimicek, Zeitlhofer, Anderer, and Saletu (1994). This algorithm captures spindle activity as duration as well as amplitude of identified sleep spindles rather than measuring the often used mean number of spindles per time (commonly referred to as spindle density). Thus, the algorithm provides spindle values (arbitrary units) reflecting the intensity or activity of the spindle process. In the following, this spindle measure will be referred to as spindle activity (SpA). Spindle detection was carried out on central electrode derivations (C3 and C4), which were offline re-referenced to contra-lateral earlobe mastoids.

SpA was analyzed for all stage 2 (30-second) sleep epochs (termed S2-SpA) as well as for all stages 3 and 4 (30-second) sleep epochs (termed SWS-SpA). In order to utilize a spindle estimate independent of time spent in NREM sleep stages (i.e., number of S2 or SWS epochs), the mean value (rather than the sum) of all spindle activity epochs was used. SpA measures were averaged over electrode sites C3 and C4. SpA was determined separately for four night quarters (referred to as SpA_1, SpA_2, SpA_3 and SpA_4) and for early and late sleep (first two night quartiles versus last two night quartiles) termed SpA_{1+2} and SpA_{3+4}.

Relative Sigma Power Measures During Sleep

Sigma band power estimates (11.5–15.9 Hz) were determined by spectral analyses for all 30-second sleep epochs. Relative sigma power during sleep was calculated using the event-related desychronization (ERD) equation from Pfurtscheller and Aranibar (1977): $ERD\% =$ [EEG power during a reference interval ($power_{ref}$) − EEG power during a test interval ($power_{test}$)/$power_{ref} \times 100$].

Three different "sleep epochs" were used as test intervals: (1) NREM epochs with spindle activity termed SpA epochs, (2) NREM epochs without spindle activity termed no-SpA epochs and (3) REM epochs. Power estimates were averaged across all no-SpA epochs, SpA epochs and REM epochs and for each electrode. The two-minute eyes closed condition recorded before the beginning of NFT served as power baseline or reference for each subject and the percentage of power change from the reference (waking with eyes closed) to the test (sleep) condition was calculated for each of the three "sleep epochs." Thus, power values throughout the paper refer to relative power estimates with respect to a reference period during relaxed wakefulness.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL). Whenever sphericity assumptions were violated in the calculated repeated measures ANOVAs, Greenhouse-Geisser corrected degrees of freedom were used. Spearman instead of Pearson correlations (one-tailed) are reported for not normally distributed variables.

RESULTS

Sleep Architecture

The percentage of time spent in each sleep stage (in relation to SPT) is depicted in Table I. A repeated measures ANOVA with the time (%) spent in each sleep stage as dependent measure and the within subject factors TRAINING (NF, PF), QUARTER (1st– 4th) and STAGE (S1–S4, REM, Wake) revealed no significant influence of neurofeedback training on sleep architecture (TRAINING: $F_{1,9} = 0.757$, n.s.; TRAINING × QUARTER: $F_{3,27} = 0.386$, n.s.; TRAINING × STAGE: $F_{5,45} = 0.303$, n.s.; TRAINING × QUARTER × STAGE: $F_{15,135} = 0.668$, n.s.).

Table I. Sleep Parameters After Neurofeedback and Pseudofeedback Training

	NFT		PFT	
	M	*SD*	*M*	*SD*
SPT	449.50	29.31	456.55	23.63
Seff	91.27	4.05	92.43	4.24
S1	6.10	(2.66)	6.70	(3.52)
S2	50.00	(7.76)	50.30	(7.76)
S3	8.60	(4.43)	9.80	(5.44)
S4	8.90	(6.22)	7.60	(5.23)
REM	22.30	(5.61)	22.20	(7.00)
W	2.60	(3.04)	2.80	(1.87)
MT	1.90	(0.60)	1.90	(0.87)

Note. Sleep stages are expressed in percentage of sleep period time (SPT). M, mean; SD, standard deviation; Seff, sleep efficiency (min); S1–S4, sleep stage 1–4; REM, rapid eye movement; W, wake; MT, movement time.

Likewise, there were no significant differences between training types in classical sleep parameters like total sleep time, wake after sleep onset, or movement time.

As revealed by a paired-sample t-test, there were also no significant differences between the two conditions in drowsiness scores assessed before ($t_{10} = 0.456$, n.s.) as well as after sleep ($t_{10} = 0.173$, n.s.).

Neurofeedback Effects

Immediate Neurofeedback Effects on EEG

To evaluate whether NFT induced immediate changes in EEG parameters, a repeated measures ANOVA with EEG band power during resting eyes open as dependent measures and the within subject factors TRAINING (NF, PF), TIME (pre-training, post-training), BAND (theta, L-1 alpha, L-2 alpha, U-alpha, sigma) and ELECTRODE (Fz, C3, Cz, C4, Pz, O1, O2) was calculated. Analyses revealed no significant results, indicating that NFT as compared to PFT had no significant influence on EEG parameters measured during an eyes open rest condition immediately following feedback training. As depicted in Fig. 2, however, a (statistical non-significant) increase in slow frequencies (4–10 Hz) post-neurofeedback training is noticeable. Altogether, though, this still violates our hypothesis that the trained *sigma band* increases post-neurofeedback training.

Neurofeedback Effects on Sleep Spindle Activity

To find out whether NFT influenced subsequent spindle activity, a four-way ANOVA with spindle activity as dependent measures and TRAINING (NF, PF), STAGE (S2, SWS) and QUARTER (1st– 4th) as within subject factors was calculated. Neither a significant main effect for TRAINING ($F_{1,9} = 0.228$, n.s.) nor any significant interactions with the factor TRAINING (TRAINING × STAGE: $F_{1,9} = 1.496$, n.s.; TRAINING × QUARTER: $F_{3,27} = 0.520$, n.s.; TRAINING × STAGE × QUARTER: $F_{3,27} = 0.115$, n.s.) could be
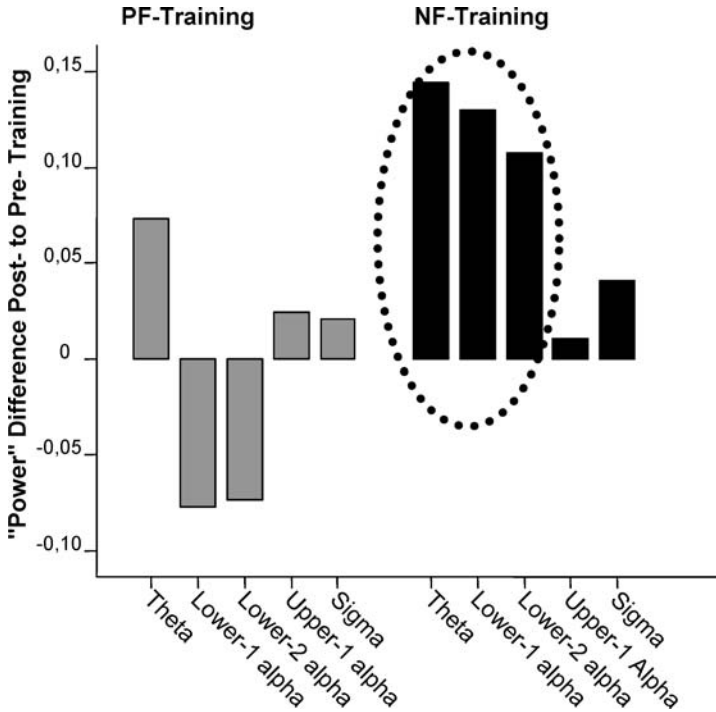
Fig. 2. The spectral power differences between pre-training and post-training session (at recordings site Cz) for both pseudofeedback (PF), as well as neurofeedback (NF) are depicted. Please note that while visually there appears to be an increase in slow frequencies (from theta to lower-2 alpha, 4–10 Hz) after sigma neurofeedback training (dotted circle) these differences do not reach statistical significance. Theta (3.9–5.9 Hz); Lower-1 alpha (5.9–7.8 Hz); Lower-2 alpha (7.8–9.8 Hz); Upper-1 alpha (9.8–12 Hz); Sigma (11.5–15.9 Hz).

revealed. It can thus be assumed that the two types of trainings performed before each experimental night had no differential effect on the amount of SpA exhibited during subsequent sleep.

Neurofeedback Effects on Sigma Band Power During Sleep

A three-way ANOVA with relative sigma power as dependent measure and with TRAINING (NF, PF), EPOCH (SpA, no-SpA, REM) and ELECTRODE (Fz, C3, Cz, C4, Pz, O1, O2) as within subject factors was calculated in order to investigate whether the type of neurofeedback training did have an impact on spindle frequency band power during sleep. The ANOVA revealed significant main effects for EPOCH ($F_{2,18} = 15.476$, $p < 0.001$) and ELECTRODE ($F_{6,54} = 28.671$, $p < 0.001$). Furthermore, there was a significant TRAINING × EPOCH × ELECTRODE interaction ($F_{12,108} = 1.936$, $p = 0.038$). Although post-hoc t-tests failed to reach significance, trends towards enhanced sigma power (during NREM sleep) on electrode site Fz and Cz after NFT as compared to PFT are evident (cf. Fig. 3).
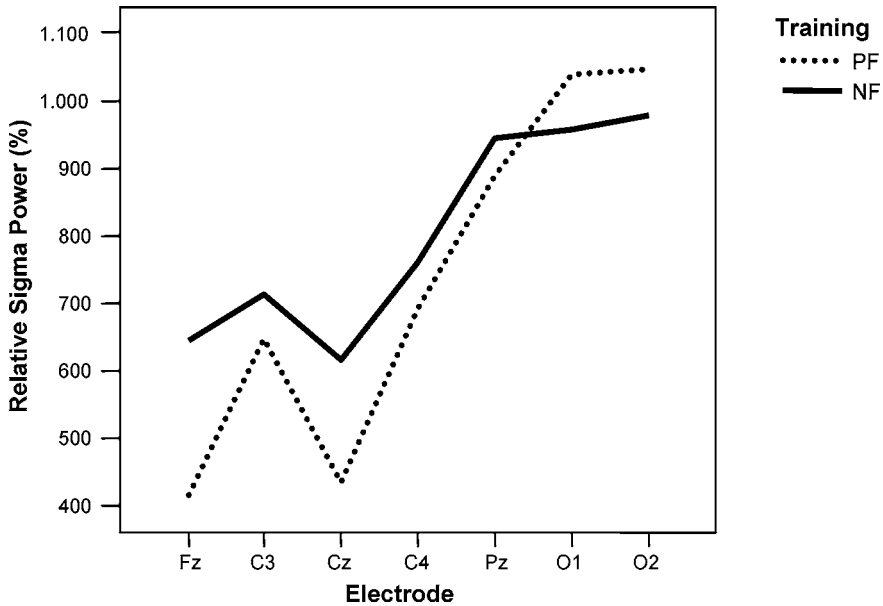
Fig. 3. Relative sigma band power (11.5–15.9 Hz) during NREM sleep is shown after PF and NF training. Note that in sleep relative sigma power at frontal and central recording sites is enhanced after sigma neurofeedback training. Relative sigma power is given as percent increase from a 2-min eyes closed resting condition (before training) to all NREM epochs containing spindle activity (SpA epochs).

Neurofeedback Effects on Memory Performance

For revealing possible NF effects on memory performance, a two-way ANOVA with memory performance scores as dependent measures and TRAINING (NF, PF) and TIME (pre-sleep, post-sleep) as within subject factors was calculated. The ANOVA revealed no significant results (TRAINING: $F_{1,10} = 0.029$, n.s.; TIME: $F_{1,10} = 0.033$, n.s.; TRAINING $\times$ TIME: $F_{1,10} = 0.002$, n.s.), indicating that our neurofeedback training did neither have immediate effects on memory nor did it influence the overnight change in memory performance.

Since NFT/PFT had no effects on the measured sleep parameters, on spindle activity or memory performance, the data from the two experimental nights (i.e., after NF and PF training) were collapsed for further statistical analyses.

Memory Performance

Table II presents memory performance scores separately for NFT and PFT sessions as well as collapsed over both experimental sessions (total). As measured by a paired-sample *t*-test, evening and morning memory performance did not differ significantly ($t_{10} = -1.82$, n.s.).

Based on the overnight change in memory performance, subjects were categorized into two different improvement groups, one with subjects who were able to improve

Table II. Memory Performance Scores Grouped by Feedback Training Condition

	NFT	PFT	Total
pre sleep	41.56(20.32)	40.85(21.63)	41.25(19.20)
post sleep	41.82(19.73)	40.97(22.31)	41.39(19.64)

Note. Values represent mean (SD) of correctly recalled words in percent for the evening (pre sleep) and morning testing session (post sleep).

their performance on the paired-word recall test overnight (termed memory improver) and another one with subjects who's memory performance did not improve or worsened overnight (termed memory non-improver). As a cut-off score for group categorization an overnight improvement of at least 0.5% was used [non-improver $\leq 0.5\%$ overnight improvement < improver; non-improver ($n = 5$): $M = -2.00\%$ ($\pm 2.00\%$); improver ($n = 6$): $M = 1.93\%$ ($\pm 1.33\%$)]. Based on the overall percentage of correctly recalled words, subjects were also categorized into two memory performance groups. Therefore, the mean performance scores of the pre sleep (evening) and the post sleep (morning) testing sessions were used as a measure of absolute memory performance. The median of memory performance among subjects was then used as cut-off score (low $\leq$ median < high), resulting in the two performance groups, one with high absolute memory performance [termed good performer: $M = 56.16\%$ ($\pm 10.66\%$)] and another one with low absolute memory performance [termed bad or poor performer: $M = 29.01\%$ ($\pm 16.08\%$)].

Sleep Spindle Activity and Declarative Memory Performance

A two-way ANOVA with SPA as dependent measures and the within subject factors STAGE (S2, SWS) and QUARTER (1st– 4th) revealed a significant main effect for STAGE ($F_{1,10} = 57.315$, $p < 0.001$) as well as for QUARTER ($F_{3,30} = 7.193$, $p = 0.001$) and a significant STAGE $\times$ QUARTER interaction ($F_{3,30} = 10.986$, $p < 0.001$). The significant main effect for STAGE indicated that on average S2-SpA during all quartiles of sleep was significantly higher than SWS-SpA. As revealed by post-hoc t-tests, the significant main effect for QUARTER indicated that generally spindle activity in the last quarter was lower as in all the other quarters.

In order to find out whether memory improver differ in spindle activity from memory non-improver, a one-way ANOVA with SpA values as dependent measures and the between subject factor IMPROVEMENT GROUP (improver vs. non-improver) was calculated. The ANOVA revealed no significant results, but a near significant result indicated that memory improvers as compared to memory non-improvers even tend to exhibited less SpA during late SWS ($F_{1,9} = 4.743$, $p = 0.057$). Pearson product-moment correlations calculated between SpA in each quartile of sleep and the amount of overnight change in memory performance, however, revealed no significant results.

Last but not least, it was tested whether there are general differences in spindle activity between "good" and "bad" memory performers. Therefore, a multivariate ANOVA with
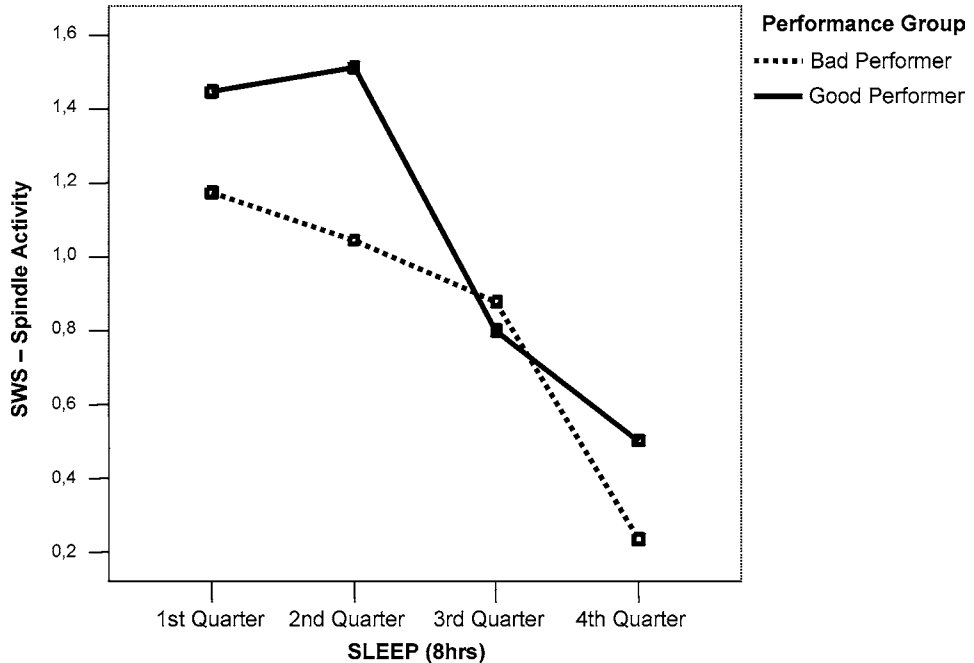
Fig. 4. Mean amount of SWS-SpA for the two performance groups. The mean amount of SWS-SpA during the four quartiles of sleep is depicted separately for good and bad memory performer. During the second quarter of sleep good memory performer exhibit significantly higher SWS-SpA as compared to bad memory performer.

quarterly SpA values ($SpA_1 - SpA_4$) as dependent variables, the between subject factor PERFORMANCE GROUP (good performer vs. bad performer) and the covariate time (%) spent in NREM sleep stages (S2, SWS) was calculated. The ANOVA revealed a significant between subject effect for $SWS-SpA_2$ ($F_{1,7} = 5.832, p = 0.046$). Post hoc independent sample t-tests revealed a trend towards higher $SWS-SpA_2$ in good memory performers ($t_9 = -2.038, p = 0.066$). Note that "good" memory performers did have a surplus of 33.33% in spindle activity values during slow wave sleep as compared to "bad" memory performers (cf. Fig. 4).

The relation between absolute memory performance and SpA was further delineated by calculating correlations between SpA and overall memory performance. Memory performance proofed to be positively correlated with SpA_{1+2} ($r_{11} = 0.59, p < 0.05$). When calculating correlations separately for S2- and SWS-SpA, one can see that this positive correlation between early SpA and memory performance is mainly caused by a highly significant positive correlation between performance and early SWS-SpA ($r_{11} = 0.72, p < 0.01$; Fig. 5). Partial correlations controlling for the duration of sleep stages (S2% and/or SWS%) revealed even stronger correlations between performance and $SWS-SpA_{1+2}$ ($r_9 = 0.82, p = 0.004$) and between performance and overall SpA_{1+2} ($r_9 = 0.68, p = 0.021$) as did correlations controlling for evening tiredness ($SWS-SpA_{1+2}, r_{10} = 0.71, p < 0.01$; $SpA_{1+2}, r_{10} = 0.69, p < 0.02$, respectively).
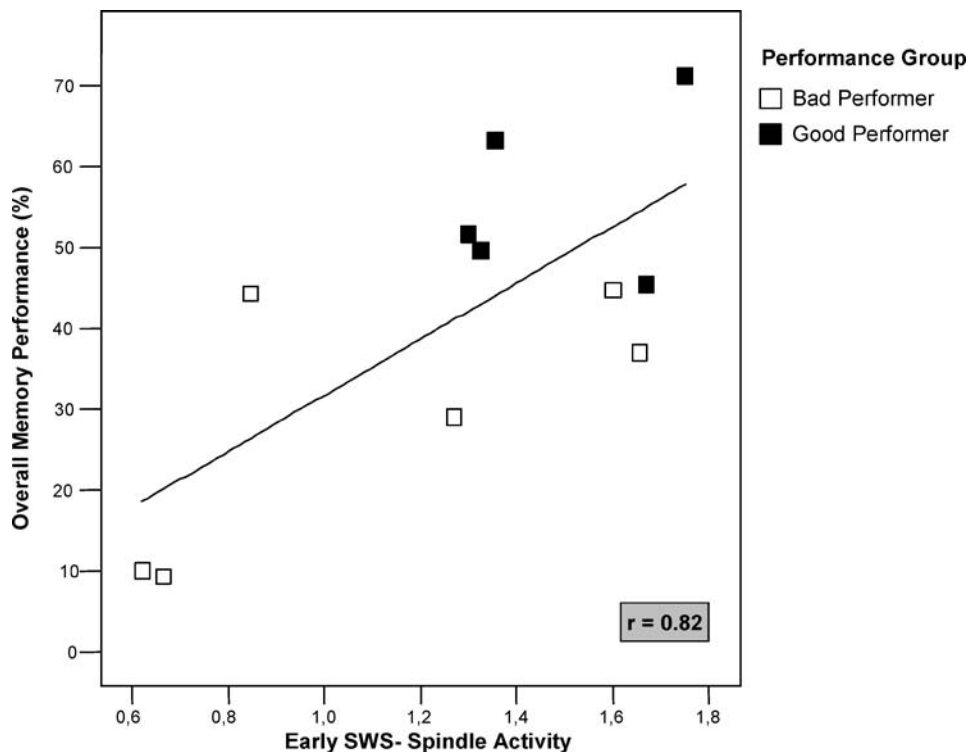
Fig. 5. Relationship between overall memory performance and early SWS-SpA. Subjects categorized as good overall memory performer (black squares) generally exhibited more SWS-SpA during the first half of the night as compared to bad memory performer (white squares).

DISCUSSION

Spindle Neurofeedback Training

Training subjects to enhance their sigma frequency did neither result in enhanced sleep spindle activity during the subsequent night nor in any overnight changes in memory performance. The data are therefore not able to replicate early findings by Sterman, Howe, and McDonald (1970) that conditioning of EEG 12–14 Hz activity (in cats over 2–4 weeks) leads to a facilitation of spindle burst sleep. However, note that the NFT protocol in the present study was only composed of 4 blocks á 10 minutes which is not comparable to the much more intense training protocol used by Barry Sterman. Most probably, a much more extensive training would have been required to also achieve enhancement in nocturnal human sleep spindle production. Furthermore, training was done on sigma band power (11.6–16 Hz) and not directly on sleep spindles which are known to be a highly stable and robust feature of human sleep (Werth et al., 1997; De Gennaro et al., 2005). The lack of detectable effects of NFT on sleep spindle production is also not surprising in the light of the finding that even during a resting condition (eyes open rest) immediately after NFT no alterations in EEG parameters could be revealed.

We also would like to point to several potential NFT shortcomings which might have obscured our training effects. On one hand, NFT at C3/C4 electrodes might have been better suited than the utilized Cz electrode as those are somewhat better located over the human somatosensory areas. Instead of the used picture completion feedback 'game,' very simple and more instantaneous feedback which also indicates to the subjects whether they train in a wrong direction (providing additional negative feedback) might have helped to get better NFT outcomes. It is also quite possible that shorter but more frequent blocks of training (e.g., 10 × 4 minutes) might have been more beneficial for training purposes. Last but not least it is unknown whether NFT after (and directly before sleep) rather than before learning word-pairs might not have had better effects. In the course of the experiment we often heard from subjects that they got quite drowsy after training which is of course sub-optimal if there is still information to encode thereafter. Thereby, our sigma NFT might have obscured our behavioral results with efficient NFT resulting in tiredness and in turn interfering with the motivation and/or the cognitive capability to engage in the memory task. It can be discussed whether the late time of day the sigma neurofeedback took place made it a more tiresome than relaxing intervention. However, the spectral band power increase from theta up to lower-2 alpha bands (7.8–9.8 Hz) immediately after neurofeedback training (cf. Fig. 2) could just as well be interpreted in terms of successful relaxation after NF training. For now, we feel unable to resolve this interesting and important question. Please also note that these increases after NFT did not reach statistical significance which might also be seen in the light of the limited sample size.

Interestingly, there was, however, a trend towards enhanced sigma band power on frontal and central electrode sites during NREM sleep after only four ten-minute sessions of NF as compared to PF training (cf. Fig. 3). It can thus be speculated that subjects might have been, in some way, able to gain some control over their EEG brain activity (i.e., sigma band power) and that changes in EEG components according to the training protocol were to a limited degree still visible several hours after the training had been terminated. Moreover, it might be important to point to the fact that these changes were most visible in sleep periods were sigma is already naturally dominant (stage 2 containing spindles).

The Significance of Sleep Spindles for Aspects of Declarative Memory

Memory Consolidation

Within the last five years, the results of only few studies have implicated the occurrence of sleep spindles in the overnight consolidation of declarative memories (Schabus et al., 2004; Clemens et al., 2005). In the present study, the expected involvement of sleep spindle activity in memory consolidation processes occurring during sleep was not found. An explanation for this finding could reside in the selected memory task with only 80 word-pairs presented once. We assume that due to that encoding before sleep was rather unsuccessful and shallow. Prior studies that were able to find a positive relation between the amount of spindle activity and overnight memory change (Schabus et al., 2004) or an augmentation of sleep spindles after a declarative memory task as compared to a control task (Gais et al., 2002) used tasks of much higher cognitive strain. As sleep spindle activity is known to be a highly stable feature of individual sleep architecture (Silverstein and Levy, 1976; Werth et al., 1997; De Gennaro et al., 2005), it is conceivable that only tasks of high

cognitive demand or involvement can slightly alter this specific brain oscillation which is also supported by a recently presented study (Schmidt et al., 2006). We think that the enhancement of spindle oscillations, believed to be crucial for off-line reprocessing and overnight improvement of memories, might not have been 'triggered' in the first place in the present study. Furthermore, as mentioned previously, the cognitive and attentional strain of the 45-minute neurofeedback training that constituted an additional part of the study design might have exerted detrimental effects on subject's engagement in the memory task and did consequently hamper an initiation of sleep spindle based consolidation processes.

To our knowledge, this is the first study to directly and separately address the role of both S2-SpA and SWS-SpA in memory processes. It might be speculated that sleep spindles occurring during SWS serve different functional purposes as compared to spindles characteristically occurring during S2 sleep. It might even be speculated that the high spindle intensity during SWS in the 2nd half of the night might indicate some preceding deficit in sleep architecture or mechanisms. For example, a study from our own workgroup (Gruber et al., 2004) demonstrated that predominantly early stage 2 spindles are important for overnight declarative memory improvement. This would be in line with the general finding that early SWS rich sleep is of higher importance for declarative memories. However, for now the exact mechanisms underlying these late SWS spindle effects remain shaded in the dark.

Learning Aptitude

In contrast to the involvement of sleep spindles in memory consolidation, the present results provide further evidence that sleep spindle activity is indeed related to an individual's general (declarative) learning ability (Gais et al., 2002; Schabus et al., 2006). It was revealed that subjects who generally managed to remember many words from the paired-associate memory task exhibited significantly more SpA during early SWS as compared to subjects who showed poorer overall memory performance (cf. Fig. 4). Comparing those subjects with high early SpA and those with low early SpA indicates nearly twice as good memory performance ($51.81 \pm 12.81\%$ vs. $28.80 \pm 19.32\%$) in subjects with high early SpA (particularly during SWS). Absolute memory performance and SWS spindle activity are also linearly related as indicated by significant positive correlations (cf. Fig. 5). The average percentage of correctly recalled words correlated significantly with early SpA from both S2 and SWS. This positive relation was mainly caused by a highly significant correlation between performance and early SWS-SpA. This effect is also not caused by variations in the time spent in different sleep stages or, as controlled by partial correlations, in variations of drowsiness levels.

Taken together, these results strongly support the hypothesis that the amount of spindle activity exhibited by an individual during sleep is an indicator of general verbal learning aptitude. In other words, the considerable inter-individual variance in sleep spindle activity appears to be partly related to differences in learning capacities. The findings are in line with earlier studies demonstrating a relationship between the total number of sleep spindles/mean sigma power and intelligence scores (Fogel et al., 2001; Nader & Smith, 2001) and deviant patterns of EEG sleep spindle activity in retarded children (Quadens, 2003). Up to the present date, however, positive findings concerning the relationship between spindle activity and declarative/verbal memory have remained very scarce (Gais et al., 2002; Schabus

et al., 2004; Clemens et al., 2005). The results from this study provide robust experimental evidence that SpA is also indicative of the aptitude to learn new declarative (verbal) material. The study—although using a small sample size—extends prior findings by demonstrating that it is primarily SpA during slow-wave sleep and the first night half that serves as a marker for declarative learning aptitude. These findings are in line with more extensive data from our lab (Schabus et al., 2006) suggesting that people with high SpA differ from people with low SpA in memory (as evaluated by the Wechsler Memory Scale-revised) as well as general cognitive abilities (i.e., non-verbal reasoning abilities as tested by Raven's Advanced Progressive Matrices).

CONCLUSIONS

The study provides additional evidence for a considerable positive relationship between an individual's general ability to acquire new declarative verbal information and the amount of nocturnal sleep spindle activity. Subjects characterized by high memory performance exhibited significantly more spindle activity, especially during early periods of sleep, as compared to subjects with generally low memory performance. The results therefore support the notion that the considerable inter-individual variance in sleep spindle activity can at least partly be explained by differences in learning ability.

The study also provides preliminary evidence that after four short pre-sleep sessions of neurofeedback training in the sigma (sleep spindle) frequency range, healthy subjects are able to gain some control over this EEG activity. The trend for enhanced sigma band-power during NREM sleep after NFT as compared to PFT might indicate that possible NFT effects become more easily evident in actual frequency bands trained rather than in the associated phasic spindle activity.

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