Diminished nap effects on memory consolidation are seen under oral contraceptive use

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Abstract

Many young females take exogenous hormones as oral contraceptive (OC); a condition rarely controlled for in studies on sleep and memory consolidation even though sex hormones influence consolidation. This study investigated the effects of OCs on sleep-related consolidation of a motor and declarative task, utilizing a daytime nap protocol.

Fifteen healthy, young females taking OCs came to the sleep lab for three different conditions: nap with previous learning, wake with previous learning and nap without learning. They underwent each condition twice, once during the 'pill active' weeks and once during the 'pill free week', resulting in six visits.

In all conditions, participants showed a significant offline consolidation effect, independent of pill week or nap/wake condition. There were no significant differences in sleep stage duration, spindle activity or spectral EEG frequency bands between naps with or without learning condition.

The present data showed a significant offline enhancement in memory irrespective of potential beneficial effects of a nap. In comparison to previous studies this may suggest that the use of oral contraceptives may enhance offline memory consolidation in motor and verbal tasks per se. These results stress the importance to control for the use of OCs in studies focusing on memory performance.

Keywords: nap, sleep, oral contraceptives, procedural motor learning, declarative verbal memory, estrogen, progesterone, pill, spindles

Introduction

The evidence for a beneficial role of sleep on memory consolidation is becoming stronger [1;2]. However, several studies show diverging results including a lack of improvement following sleep [3-8]. A possible explanation for this confusion may be a disregard of additional confounding factors. For one, studies rarely control for sex, menstrual cycle or the use of oral contraceptives (OCs). The hormones estrogen and progesterone have a wide range of effects on sleep as well as on memory. On the molecular and synaptic level, estrogen positively influences the hippocampus and other memory related brain areas, by inducing a beneficial environment for memory encoding and consolidation [9;10]. On the behavioral level, it is important to distinguish between tasks in which males typically show an advantage (e.g. spatial) and tasks in which females typically show an advantage (e.g. verbal, fine motor) [11]. This has clearly been demonstrated in animal models; however results in human studies are more variable. These latter tasks are positively influenced by the hormones estrogen and progesterone. In contrast, tasks with a male advantage are affected negatively by the same hormones [12-16]. Furthermore, use of OCs influences memory encoding: Females showed enhanced verbal memory during the active OC phase [17]. Another study presented that after sleep deprivation, females in the follicular phase performed worse on different cognitive tests than females in the luteal phase or taking OCs [18]. Wharton and colleagues [19] could show by comparing different OC products that the androgenic activity in OCs influence mental rotation task performance, a typical "male" task. Not only memory, but also sleep is influenced by exogenous hormones. Females taking OCs have less slow wave sleep, increased stage 2 sleep, shorter REM onset latency and more REM sleep than naturally cycling women [20;21].

In a previous study we could demonstrate a sex and menstrual cycle effect on sleep-related memory consolidation of "female" tasks [22]. While male subjects benefitted from a

nap in verbal and in motor learning, females did so only during the mid-luteal phase with high levels of estrogen and progesterone, however not during the early-follicular phase with low levels of the respective hormones. Effects in motor learning were correlated with hormonal levels of progesterone, and effects in verbal learning with levels of estrogen in the participants. Sleep spindles showed a similar effect. Spindle activity increased upon learning in males, whereas in females it increased only during the mid-luteal phase, matching the learning behavior. Furthermore, sleep spindle density and frequency correlated with estrogen [22].

The majority of studies on human memory functions investigate healthy young subjects. At the same time many young females take OCs – around 72% of all 18-29 year old females in Germany [23] – therefore are under the influence of exogenous estrogen and progesterone. However, this condition is rarely controlled for or even regarded as potential confound.

Aim and hypothesis

To investigate if OC use in participants of sleep and memory studies may confound the outcome of these studies, we investigated the effects of OCs on verbal and motor memory consolidation during a daytime nap. A nap has been shown to be as effective for memory consolidation as a whole night of sleep for these tasks, but has the advantage of avoiding time of day or stress via sleep deprivation as confounds [24-34]. Females taking OCs underwent three conditions – a nap with learning, wake with learning, and nap without learning. Participants did so once during a pill week and once during the regularly recurring pill free week, resulting in six visits altogether. Based on our previous finding of strongest enhanced memory consolidation during the third week of the natural menstrual cycle, with highest levels of estrogen and progesterone, we expected to see a similar strong effect in the participants when taking OCs. We hypothesized that participants taking OCs would show enhanced memory consolidation in both tasks.

Materials and Methods

Participants

The participants were healthy female volunteers (n=15) aged 18-30 years taking oral contraceptives. They were recruited mainly via the local medical school and were paid for the participation in the study. All participants were first screened for psychiatric, physical, or sleep disorders with a semi-structured interview, physical examination and the Pittsburgh Sleep Quality Index [35]. Additionally, we obtained urinary-drug-screening and routine blood tests (blood cell count, electrolytes, liver and kidney function, thyroid hormones). Further exclusion criteria were: shift-work at night, a transmeridian flight, any medical treatment during the last three months, substance abuse (assessed via oral question and urinary drug screening), professional piano playing (more than 5 years intensive training), professional type writing, extreme chronotypes (scores of >70 and <30, assessed via the D-MEQ [36;37]) and regular naps (>2 nap/month). Professional pianists and typists were excluded since their baseline tapping performance would be significantly higher than the other subjects, which could affect offline improvement. All participants took one of two types of oral contraceptives (OCs): Valette (Bayer Austria GmbH Vienna/Austria) and Belara (Gruenthal GmbH Aachen/Germany). Both OCs had equal amount of estradiol (0.03 mg ethinylestradiol), and different gestagens (2 mg dienogest (Valette) and 2mg chlormadinoacetat (Belara) respectively). OC intake was for at least one year prior to the study, following the generally recommended scheme of three weeks of daily intake followed by a ‘discontinuation’ week during which menses may occur.

The participants agreed to have regular sleep patterns throughout the experiment and kept a sleep diary for each week preceding a study block. The Ethics Committee of the Ludwig Maximilian University, Faculty of Medicine, Munich/Germany, approved the research project. The experiments were undertaken with the understanding and written

consent of each subject, and the study conforms to The Code of Ethics of the World Medical Association.

Procedures

All subjects underwent 6 study days (see Figure 1): nap with learning, wake with learning, nap without learning; each of the three conditions once during the 'pill active' phase during OC intake (second week of the pill cycle) and once during the monthly recurring 'pill-free' week. The order of all 6 conditions was randomized and balanced between participants. The conditions were separated by 27d ±18 with a range of 10-86 days. In addition, in each experimental condition blood for hormonal analysis was drawn from a peripheral vein after the subjects arrived at the lab.

The nap-protocol used in this study, has been established and used previously [22;24;34]. During study days the subjects arrived at 13.00 h; they first completed the D2 Concentration test (D2) [38] and the Stanford Sleepiness Scale (SSS) [39] followed by the learning phase of a verbal paired associate task [32] and a sequential finger tapping task [40]. During the learning phase and the retest we conducted the four learning and alertness tasks in a randomized order to avoid a confounding effect of a reciprocal interaction between the tasks [41]. We had three "stations" (SSS and D2, tapping, word pairs), which resulted in 6 different orders. The subjects were pseudo randomly (so randomly but then balancing across participants with 2-3 participants having the same order) assigned one of the 6 order sequences. Subsequently at around 14.00h the subjects were informed to which condition they had been assigned: participants in the WAKE-condition (L-Wake) watched a non-emotionally-arousing movie until retest; in participants in the NAP-condition the electrodes were placed, the lights were turned off, and the subjects were allowed to sleep for

approximately 60 min (L-NAP). A 60 min nap duration was chosen matching previous studies [22;24;34] so that most subjects would have naps containing stage 2 sleep and slow wave sleep. At around 16:30 h or at least 30 min after awakening from the approx. 1-hour-nap, all subjects completed the D2 test, the SSS, and the retest, after which they returned home.

During the nap without learning condition the participants arrived at 14:00, filled out the D2 and SSS and took a nap at the same time of day (C-NAP), with polysomnographic recordings but without learning tasks. This condition consisted solely of a nap without learning to investigate changes in sleep induced by learning (C-NAP vs. L-Nap).

The participants were instructed to refrain from rehearsal of the tasks and to keep a regular sleep cycle throughout the weeks of the experiment. In addition the participants kept a sleep diary for a week preceding each study block. During this week they went to bed between 23.00h and 1.00h and woke between 7.00h and 9.00h; during the three nights prior the study day the bedtime changed to 23.00 – 24.00h and the wake-time to 7.00 – 8.00h.

Figure 1 please near here

Hormone measures

Directly after the participants arrived at the sleep lab blood was drawn for hormonal analysis. Immediately after the draw the test tubes (serum-tubes with clot activator, 7.5 ml, from Sarstedt Nuembrecht/Germany 01.1601.001) were centrifuged and transferred to the in-house lab for analysis, or refrigerated (~4°C) until analysis could be performed. Hormones – 17 beta estradiol and progesterone – were measured by electrochemiluminescence, with an Elecsys 2010 analyzer (Roche Diagnostics, Basel/Switzerland). Functional sensitivity for 17 beta estradiol was 12 pg/mL, and for progesterone 0.15 ng/mL. In our lab it was only possible

to measure the levels of endogenous hormones estrogen and progesterone and not of the exogenous OC hormones. Reported pharmacological properties and measurements for Valetta are for dienogest: maximum plasma concentration 51.6±9.5ng/ml reached in 2.4±1.4h, steady state after daily intake 1.5 fold serum levels, 96%bioavailability, 10% plasma free form, 90% bound to albumin, 9.3±1.8h half-life, 3.66±0.71L/h clearance; and for ethinyloestradiol: maximum plasma concentration reached in 1.5-4h steady state after daily intake 2 fold serum levels, 44%bioavailability, 98.5% bound to albumin, 11.7±6.5h half-life, 5mL/min/kg metabolic clearance.

Polysomnographic recording parameters

Polysomnographic data were recorded in all nap conditions; stored and analyzed with a digital recorder (Comlab 32 Digital Sleep Lab, Brainlab V 3.3 Software, Schwarzer GmbH, Munich, Germany). We recorded scalp EEG from the C3 and C4 derivations referenced against the contralateral mastoid (filtered from 0.5 to 70 Hz), and further electrooculograms (EOG) and mental/submental electromyogram (EMG), with a sampling rate of 250 Hz.

Learning Tasks

All subjects learned two tasks; one declarative (verbal) and one procedural task (motor).

The tool for declarative memory analysis was a paired associates learning task. We used paralleled standardized word lists consisting of 40 related word pairs (e.g. Nanny – Stroller), with additional 2 dummy pairs in the beginning and at the end to avoid inclusion of primacy and recency effects [32]. In the learning condition the word pairs were first presented for 5 s each, and immediately after the list presentation a cued recall followed in which the participant was asked to type each matching noun after being shown the first word of the pair. If the participant was not able to recall the right word, the correct answer was displayed. Thus

every participant saw the correct pairing twice, once in the learning phase and once during retest. This method aims to avoid differences in exposure to the learning material by differences in recall performance. Each word pair was cued once. In the retest condition (delayed recall after approx. 3.5hrs following nap / wake condition), the cue words were given once and the number of correctly known word pairs was obtained by the experimenter to compensate for spelling errors. At the training and retest condition the subject had unlimited time to respond to the cued recall. In order to measure sleep-related consolidation we used absolute change in performance from learning to retest (e.g. performance at learning 15 correct word pairs and performance at retest 20 correct word pairs resulted in consolidation measure of 5).

To test procedural motor memory, we employed a sequential finger tapping task [40]. This task required participants to press four numeric keys on an altered computer keyboard with their non-dominant hand, repeating the five element sequence (e.g. 4-1-3-2-4) as quickly and accurately as possible for a period of 30 s. Four different sequences were used in the experiments. To exclude any working memory component on the task, the numeric sequence was displayed on the screen. For every trial the computer noted the number of complete sequences achieved, the number of errors made, and the number of correct sequences typed. The learning phase consisted of twelve trials of 30 s interrupted by 20 s rest periods, while at retest the subjects had to complete four trials. As score we used the number of the correctly tapped sequences during the period of 30 s, which incorporates the accuracy and speed performance. End-training performance consisted of the average score from the last three trials of the training, while retest performance was composed of the average score from all four retest trials. To measure sleep-related consolidation, end-training performance was used as baseline and the change to retest performance was divided by the end-training performance

(e.g. performance at learning 20 correctly typed sequences per 30s and performance at retest 25 correctly typed sequences per 30s resulted in consolidation measure of 25%).

Sleep data analysis

For sleep data analysis, independent professionals scored the sleep stages using standard criteria [42]. The scorers were blind of the study design. Additionally, the EEG of the experimental naps (L-Nap, C-Nap), contra-lateral to the typing hand, underwent a spectral analysis through a fast-fourier-transformation using in-house software. The EEG was digitally filtered from 0.53 to 30 Hz (24dB/octave) after sweeps with visually identified EEG artifacts had been carefully removed. Power spectra were derived from 2 s windows, shifted for 1 s and averaged per epoch of 30 s. Frequency bands (based on summed power values) were calculated for the delta (0.53 - 4 Hz), theta (4.5 – 8 Hz), alpha (8.5 – 12 Hz), sigma (12.5 – 16 Hz), and beta (16.5 – 20 Hz) frequency range.

Sleep spindle analysis

An automated algorithm detected the sleep spindles. The algorithm first removes periods of EEG signal with muscle artifacts and strong alpha frequencies. Afterwards an individual spindle threshold is set for each channel and spindles are identified with continuous wavelet transformation. For a more detailed description of the analysis see supplementary materials. Analyzed parameter was spindle activity (SpA; mean spindle amplitude×mean spindle duration). We used SpA since it well reflects the intensity of the spindle process [43;43;44;44;45;45].

Statistical Analysis

For statistical analysis of offline memory consolidation, each an ANOVA was performed for the verbal and motor consolidation measures with the within-subject factors week (OC/OC-free) and condition (nap/wake). In addition, the change in performance from the end of the learning phase to retest after sleep or wake for both tasks was tested via paired T-tests considering a bonferroni corrected statistical threshold ($p<0.05/4$). For the polysomnographic data, we performed each a MANOVA with repeated measures of (a) the duration of sleep stages, (b) the EEG frequency bands and (c) spindle activity, with within-subjects factors "naps" (factor levels L-NAP and C-NAP) and week (OC/OC-free). The alertness data (D2, SSS) and the absolute end-training performance for both learning tasks were analyzed with each a MANOVA with within-subjects factors week (OC/OC-free) and condition (nap/wake). The hormone values of progesterone and estrogen were correlated with overnight change in memory performance. Alpha was set at 0.05.

Results

There was no week or condition effect on the absolute end-training performance of both the declarative and the motor task (declarative: condition (L-Nap vs. L-Wake): $F_{1,14}$=.13, P>.7; week (OC/OC-free): $F_{1,14}$=.001, P>.9; condition*week: $F_{1,14}$=.11, P>.7; motor: condition (L-Nap vs. L-Wake): $F_{1,14}$=.44, P>.8; week (OC/OC-free): $F_{1,14}$=.08, P>.7; condition*week: $F_{1,14}$=2.39, P>.1), demonstrating that all subjects started from comparable baseline levels. There was a practice effect (baseline/retest) on the concentration task but not on the sleepiness scale: MANOVA with factors test (baseline/retest), week (OC/OC-free), condition (L-Nap/L-Wake), and their interactions showed a significant effect for test (all $F_{2,12}$=8.74, P=0.005, D2: $F_{1,13}$=14.55, P=0.002, SSS: $F_{1,13}$=2.02, P>0.1) but no interaction or factor effects for week and condition (all P>0.05). An ANOVA with the factors test (baseline/retest), week (OC/OC-free), condition (L-Nap/L-Wake), and their interactions showed a significant effect for test (verbal learning: $F_{1,14}$=69.019, P<0.001, motor learning: $F_{1,14}$=43.404, P<0.001) but no interaction or factor effects for week and condition (all P>0.05). For both tasks a significant increase from end-training performance to post nap/wake retest performance was seen in all 4 conditions (all P<.008 with corrected threshold at P<.0125, see Table 1). The ANOVAs for motor learning (condition (L-Nap/ L-Wake): $F_{1,14}$=.031, P>.8; week (OC/OC-free): $F_{1,14}$=2.282, P>.15; condition*week: $F_{1,14}$=.355, P>.5) as well as verbal learning (condition (L-Nap/L-Wake): $F_{1,14}$=.016, P>.9; week (OC/OC-free): $F_{1,14}$=.225, P>.6; condition*week: $F_{1,14}$=1.377, P>.2) showed no significant differences in the offline consolidation measures between any of the different conditions (see Figure 2). This remained the same if relative instead of absolute increase was used for verbal learning (condition (L-Nap/L-Wake): $F_{1,14}$=.363, P>.5; week (OC/OC-free): $F_{1,14}$=.090, P>.7; condition*week: $F_{1,14}$=.322, P>.5). There was no significant offline change in errors in the motor task or a condition/week effect on errors indicating that the increase in general motor performance was

due to an increase in speed (ANOVA with factors test (baseline/retest), week (OC/OC-free), condition (L-Nap/L-Wake), and their interactions showed no interaction or factor effects for test, week and condition (all P>0.45).

Figure 2 and Table 1 please near here

All subjects fell asleep during their naps with an average sleep duration of > 60 min (with average light-out of ~90 min). Polysomnographic data revealed allocation of sleep stages with mainly stage 2 sleep and SWS, and additionally a small amount of REM sleep in some subjects. There was no effect of conditions (C-Nap/L-Nap/OC/OC-free) on sleep stage distribution or data from spectral analysis of the sleep EEG (see Table 2). There were no condition or OC phase effects on sleepiness and concentration at the learning phase or at retest (condition (L-Nap vs. L-Wake): $F_{4,10}$=.899, P>.5; week (OC/OC-free).): $F_{4,10}$=1.566, P>.2; condition*week: $F_{4,10}$=.552, P>.7). No significant effect of week or condition on spindle activity could be found (condition: $F_{1,14}$=.019, P>.8; week: $F_{1,14}$=.227, P>.6; condition*week: $F_{1,14}$=.028, P>.8); this remained true for other spindle measure in sleep stage 2 as well as considering all NREM (see suppl. materials). Endogenous hormonal levels of all six conditions are presented in Table 3.

Table 2 and 3 please near here

The change in tapping performance and word pairs did not correlate significantly with the amount of each sleep stage (stage 2, SWS, REM, TST) or with sleep spindle activity during the naps (all 2-tailed, r<.3, P>.15). We did find a significant positive correlation

between change in word pairs and endogenous estrogen across all conditions (1-tailed, r=.358, P<.003). However, the correlation seemed dominated by one outlier. After exclusion of the outlier the correlation still was significant, but only 1-tailed (1-tailed, r=.235, P<.05). The change in word pairs did not correlate with progesterone and the change in tapping did not correlate with any of the hormone values (all 2-tailed, r<.14, P>.25).

Sample size and power calculation are presented in the supplementary materials.

Discussion

This study investigated the effects of oral contraceptives (OC) on offline memory consolidation (= all consolidation processes, which occur when the person is not actively engaged in learning). Participants taking a contraceptive pill performed at a significantly higher level during retest four hours after the learning session. This improvement occurred regardless of an interim nap of roughly 60 minutes or staying awake in the same period. This finding also occurred irrespective of OC week (active OC uptake or (monthly) OC-free week).

In a previous study utilizing the same tasks and procedures, we had investigated the effects of the menstrual cycle on memory consolidation [22]. In the menstrual cycle study the participants started at a similar behavioral baseline as in the present study, however only the females in the nap condition during the mid-luteal phase (high with estrogen) managed to increase their performance by 7 word pairs, while all other groups/conditions (men or females in the follicular phase) only knew roughly 4 word pairs more during the retest (for visual comparison see supplementary figure 1). This might indicate that the increase of 8 word pairs in the current study – regardless of OC phase or nap/wake condition – may represent a comparable strong improvement, possibly connected to the exogenous and endogenous hormonal levels. As seen in the previous study [22], we again found a correlation between endogenous estrogen and change in word pairs. Regrettably only endogenous and not OC hormone levels could be measured in our lab, since most likely the strong improvement was induced by the endogenous as well as the exogenous hormones.

Independent of the length or content of the word lists used, sleep related effects on verbal memory usually seem to occur in a similar range. Lists with 40 word pairs (based on [32] as used here) are the most common tool in studies investigating effect of sleep on declarative memory. Irrespective of the length of sleep (nap or whole night condition), the

offline change reported is usually in the range of -2 to +5 word pairs [24-32]. Only the studies by Tucker and colleagues [33;34] reported a higher offline change of around 8 word pairs as was similarly found in the present data on OC use, as well as during the luteal phase in women [22]. However, it would be beneficial to replicate this study with a whole night of sleep to confirm that the length of sleep does not influence offline change.

A similar effect is seen in the tapping performance. On average, participants increase their performance by roughly 0-5% after wake and 10-30% after sleep [22;27-31;40;46-55]. Regardless of OC phase or nap/wake condition, the increase reported here was 10 to 17% similar to previous data seen only after sleep.

A positive effect of OCs on memory encoding has been shown previously. Participants taking OCs performed better at a verbal task during immediate testing – not delayed as in this study – than natural cycle women [17;17]. In this study we did not find an effect of OC-phase (active OC intake or OC-free interval) on memory. While some studies do report a OC-phase effect [17;56], other studies do not find such an effect [57-59]. It does not seem too surprising that there was no phase effect on memory, if one considers the range of absolute hormone values. While our subjects did show a significant rebound-effect in estrogen during the OC-free week, the values of endogenous estrogen consistently remained low in comparison to women with normal menstrual cycles (ranges: OC 9-50 pg/ml, menstrual cycle 55-155 pg/ml see [22]) while exogenous estrogen levels were most likely high.

There seemed to be no additional benefit of a nap on memory consolidation in this study. There are different possible explanations for this finding. One likely assumption is that the hormones in the OCs boost the consolidation in such a way that no additional benefit of sleep was possible. Another possibility is that a ceiling effect was reached in the tasks themselves. Further, it is also possible that estrogen increased plasticity during encoding and that increased encoding masked or influenced the effects of sleep on consolidation. Especially

since it has been reported previously that pre-sleep performance levels can influence sleep related benefit [60] .

A wide range of effects of estrogen and progesterone on the hippocampus and other brain areas important for memory has been observed. An estrogen influence on plasticity was evidenced after exogenous estradiol administration in ovariectomised rats by increases in neurogenesis [61], neural network connectivity and synaptic transmission [9]. Furthermore, estrogen increases glucose transport, glycolysis and mitochondrial function to provide the ATP necessary for energetic demand as seen in non-human primates and after exogenous estradiol administration in ovariectomised rats [9]. Estrogen affects cell morphology, synapse formation, signaling and excitability in the hippocampal formation [62-64]. In the hippocampus and the medial prefrontal cortex estrogen increases dendritic spines, and an increase in spine density has been associated with learning and memory [9;64]. Estrogens upregulate adult hippocampal neurogenesis and synaptic protein levels in the hippocampus as well as enhance synaptic NMDA receptor current and the magnitude of long-term potentiation, a cellular correlate of learning and memory [14-16].

It seems that in humans as well as rodents estrogen affects different types of memory differently [11;65;66]. In general, memory can be divided into tasks in which females show an advantage (fine motor, verbal, object location etc.) as well as tasks in which males show an advantage (mainly spatial) [67-70]. “Female” tasks seem to be positively influenced by the hormones estrogen and progesterone, while “male” tasks seem to be negatively influenced [12-16]. In both types of tasks a menstrual waxing- and waning effect can be seen. On tasks in which women typically score better than men, women perform better during mid-luteal phase (high estrogen and progesterone) than within menstrual phase (low estrogen and progesterone). On tasks in which men typically outperform women, women do best during menses [13;71;72].

Caveats

It is important to note that this study does not intend to advertise OCs as neuroenhancers. For one, we did not perform a placebo controlled, double blind cross-over study, which would be needed to be able to attempt this conclusion. Secondly, our sample size may also have been too small to detect more subtle effects, however we did not even see a trend in the data and the sample size is comparable with most studies investigating sleep related consolidation. Thirdly, we did not investigate the effect of OC use on "male" learning tasks. Since female hormones actually exhibit negative effects on memory tasks in which males outperform females, the offline consolidation of those tasks may actually be reduced by OC use. Instead this study attempts to underline the importance to acknowledge OC use as an influencing factor in sleep and memory research, which should be controlled or manipulated. A further caveat is that we did not perform an adaption nap, which could have influenced the result.

Conclusion

We could show that female participants taking OCs experienced a significant and rather large improvement during offline consolidation in a verbal and a fine motor task independent of nap/wake condition. It is tempting to speculate that this already strong enhancement in comparison to other studies was caused by the OCs and masked any potential sleep effects.These results are important pilot findings and should be confirmed with a placebo controlled, double blind cross-over study. But they do point towards the importance to control for OC use in studies investigating memory effects. Such effects may also hold responsible for some of the discrepancies in previously published results.

Acknowledgments

We thank our participants and the sleep lab team for expert data handling.

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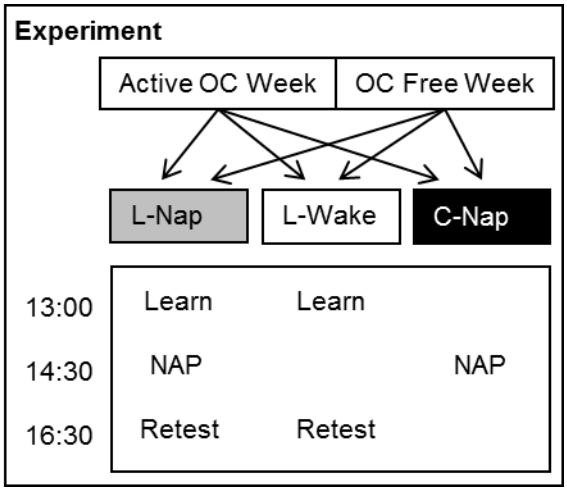

Figure 1: Study design: All participants underwent 6 study days: nap with learning (L-Nap), wake with learning (L-Wake), nap without learning (C-Nap); each of the three conditions once in the active OC week (second week of the three pill weeks) and once in the OC free week. The order of all 6 conditions was balanced across participants.

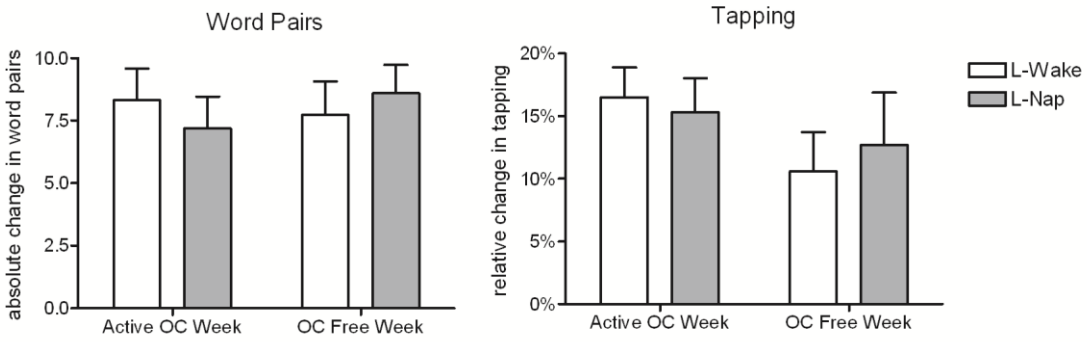

Figure 2: Change in declarative (absolute change in number of words, with SEM, left) and motor (relative change in correctly tapped sequences during 30 sec trial, with SEM, right) performance from the learning phase (13:00 h) to the retest in the afternoon (16:30 h), separated in the groups with (L-Nap) and without (L-Wake) a nap between learning and retest. There was no significant difference between the conditions.

Table 1: Absolute task performance at the end of training and at retest after either nap (L-NAP) or wake (L-Wake) during the active OC and OC free week (mean with SD). For both tasks a significant increase in performance was seen after the offline period regardless of nap/wake condition or OC phase. All tests were significant after correction for multiple comparisons for each task ($p<0.0125$).

		Active OC week		OC free week	
		L-NAP	L-Wake	L-NAP	L-Wake
Word Pairs	end-training	27.2 ± 7.9	27.0 ± 7.5	27.6 ± 7.7	26.7 ± 5.5
	retest	35.5 ± 4.8	34.2 ± 3.6	35.3 ± 3.6	35.3 ± 3.3
	statistics	T_{14}=6.6; P<.001	T_{14}=5.7; P<.001	T_{14}=5.8; P<.001	T_{14}=7.6; P<.001
Tapping	end-training	18.2 ± 3.9	18.7 ± 3.9	18.9 ± 2.9	18.3 ± 3.5
	retest	21.2 ± 4.6	21.6 ± 5.0	20.9 ± 3.7	20.4 ± 3.4
	statistics	T_{14}=7.3; P<.001	T_{14}=5.7; P<.001	T_{14}=3.5; P=.003	T_{14}=3.1; P=.007

Table 2: Sleep stage duration (minutes with SD) and power in the EEG frequency bands (μV^2 with SD) of the nap with (L-NAP) and without (C-NAP) previous learning session. Data are reported as obtained during the OC week and during the OC free week. There was no significant difference between the two conditions and the two weeks.

	Active OC week		OC free week		Statistics
	L-NAP	C-NAP	L-NAP	C-NAP	Repeated Measures MANOVA
(min)					
S1	11.3 ± 7.5	14.3 ± 10.9	11.1 ± 9.2	11.5 ± 8.0	Nap: $F_{5,10}$=.720; p>.6
S2	31.2 ± 18.3	26.5 ± 13.1	28.3 ± 13.6	30.3 ± 9.2	Week: $F_{5,10}$=.535; p>.7
SWS	20.0 ± 10.4	20.2 ± 12.4	23.1 ± 20.3	19.3 ± 13.1	Nap* Week: $F_{5,10}$=1.264; p>.3
REM	2.6 ± 5.8	1.8 ± 3.6	2.9 ± 4.9	5.3 ± 6.8	
TST	66.1 ± 14.6	62.9 ± 14.3	65.6 ± 24.0	66.5 ± 12.7	
(μV^2)					
Delta	550 ± 208	687 ± 452	605 ± 490	581 ± 288	
Theta	87 ± 35	102 ± 54	79 ± 24	90 ± 42	Nap: $F_{5,10}$=1.255; p>.3
Alpha	52 ± 24	63 ± 45	49 ± 26	51 ± 23	Week: $F_{5,10}$=1.141; p=.4
Sigma	23 ± 13	27 ±17	20 ± 8	23 ± 10	Nap* Week: $F_{5,10}$=.537; p>.7
Beta	8 ± 3	9 ± 7	8 ± 5	8 ± 4	

Table 3: Endogenous hormone values (mean with SD in pg/ml) for all conditions: nap with learning (L-NAP), wake with learning (L-WAKE), nap without learning (C-NAP).

	17-Beta Estrogen			Progesterone		
	L-Nap	C-Nap	L-Wake	L-Nap	C-Nap	L-Wake
OC week	12.0 ± 8.2	9.5 ± 4.4	8.9 ± 4.2	.30 ± .16	.25 ± .18	.28 ± .18
OC free week	36.0 ± 49.0	48.6 ± 41.9	25.8 ± 23.4	.32 ± .19	.28 ± .20	.30 ± .16
statistics	T_{14}=1.9; P=.08	T_{14}=3.5; P<.005	T_{14}=2.8; P<.02	T_{14}=.59; P>.5	T_{14}=1.2; P>.25	T_{14}=.72; P>.45