SLEEP-DEPENDENT EMOTION REGULATION

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1. Financial reports

Granted by BIAL: 31 000€

Dispatched as follows

EEG system ➔ 29 370 €

Meeting with the Japanese team: 1 270 €

Fees for participants: 50€ * 7 "night" participants (because our protocol has been performed on 3 nights rather than 2 nights): 350€

2. Research reports

- **Objective.**

Recent neuroimaging studies support that emotion regulation could be strengthened by sleep. First, MRI findings (Yoo et al., 2007; Walker et al., 2011) have demonstrated that REM sleep decreases amygdala reactivity to intrinsically emotional stimuli, previously experienced. Second, one study (Pace-Schott et al., 2009) suggests that sleep reduces affective impact of an emotional learning. However, it remains unclear whether brain activities related to emotional regulation can be identified during sleep. The main goal of our work was to identify neural activities during sleep as the cause of reduced emotional reactivity for a new emotional learning.

- ## Methods.

a. participants

Two groups, an experimental one ("sleep" group; 13 participants, 10 females; age: 25± 4.5) and a control one ("day" group; 15 participants; 10 females; age: 23± 4.1 years) performed our protocol. All controls had no history of neurological or sleep disorder, and none was being treated with medications at the time of the study. All subjects were right-handed and native French speakers. They gave their written consent to the study after detailed information was provided to them, and the study was done in-line with the Declaration of Helsinki following approval by the Regional Ethics Committee.

b. Sleep recording and analysis

For all subjects, sleep was recorded in the sleep laboratory for 3 consecutive nights. The first night was performed to accustom the subjects to the experimental conditions including placement of the electrodes. Only data from the next nights were analyzed. Polysomnographic sleep acquisition was performed by BioSemi Acquisition System (granted by BIAL) and included continuous recordings of EEG, electrooculogram (EOG), electromyogram (EMG) recorded at chin, and electrocardiogram (ECG). EEG activity was recorded with derivations at left (T3/O1) and right (T4/O2) temporo-occipital areas of the extended 10-20 international system (Nuwer et al., 1999), using Ag/AgCl electrodes with a vertex ground and a right ear reference. The impedance for all the electrode sites was kept below 10 kΩ. The EEG filter bandpass was 0.03 to 35 Hz and was digitized at 125 Hz.

Three additional electrodes were placed at the outer canthus and supraorbitally to the right eye with a bipolar recording for EOG activity. Two of us (YK, PH) scored sleep data i) to monitor quality of the recordings throughout the night, ii) to classify sleep stages following the criteria defined by Rechtschaffen and Kales (Rechtschaffen & Kales, 1968) and iii) to screen artifacts. Individual EEG traces were manually inspected for any remaining eye movement, ECG, EMG or movement-related artifacts. In addition, the potential ECG contribution to the EEG was eliminated off-line by submitting the data to an ECG correction algorithm using source separation (fast ICA algorithm, (Hyvarinen & Oja, 2000). Lastly, four EEG epochs (16 s) before and after

each change of sleep stage were also rejected to limit the uncertainties at the level of transition of sleep stage.

Computerized spectral analysis was performed using Fast Fourier Transformation (FFT) on the all-night filtered EEG after elimination of epochs with artifacts. Before computing the FFT, the data were tapered with Hamming window. The FFT was computed on artifact-free epochs of 512 points corresponding to a 4s duration. The FFT was realized for each sleep on a total number of epochs corresponding to the maximal number of artifact-free epochs observed in all subjects. Consequently, the total sample size was different for each stage but similar for all subjects. We obtained respectively for light sleep (stages 1+2): 570 epochs, SWS (stages 3+ 4): 247 epochs, REM: 154 epochs. Frequency bands were defined as follows: delta (1.5-4 Hz), theta (4-7.5 Hz), alpha (7.5-12.5 Hz), beta (12.5-30 Hz). Two parameters were measured from the spectral analysis for each frequency band. The relative power (RP, percentage) was calculated by dividing the absolute power in each frequency band by the total power of the whole spectrum. In particular, we preferred using relative power rather than absolute one because the former is not affected by the electric properties of head volume conductor. Moreover, RP recorded at a particular area is more strongly associated with local EEG than absolute power (Claus et al., 2000). The second parameter recorded for each frequency band was the mean frequency (MF) which is the weighted sum of spectral estimates, divided by absolute power in the frequency band.

c. Emotional responses

Emotional responses for each experimental session were assessed at two different levels: physiological responses and emotional feelings.

Physiological responses: physiological response to emotional and neutral stimuli was assessed by mean of galvanic skin responses (GSR). Each session began with the placement of electrodes and a 5-min rest period for electrodermal recording stabilization. GSR was recorded with BioPac System at the left hand in conductance by the constant-voltage method (0.5 V). Ag/AgCl electrodes (8 mm diameter of active area) were filled with 0.05 M NaCl electrolyte, following recommendations by Fowles et al. (1981). Electrodes were attached to the medial phalanx of the second and third

fingers by means of double-sided adhesive stamps and connected to isolated skin conductance coupler. The coupler was linked to a microcomputer whose software allowed visualization, storage and analysis of electrodermal responses.

Emotional feelings: Participants reported their emotional states via six items taken from the Differential Emotions Scale (Philippot, 1993), which measured their degree of happiness (1. amused, joyful, merry; 2. warmhearted, gleeful, elated), anger (3. angry, irritated, mad; 4. "disgusted" , turned off, repulsed) and fear (5. fearful, scared, afraid; 6. anxious, tense, nervous) on a 5-point Likert scale (1 = not at all / 5 = completely).

d. Protocols

The control condition (CC) was performed the first day (or after the first night) and was followed by the conditioning protocol (CE) the following day. The two conditions are similar but in the conditioning protocol, pictures are fearful. We present below the conditioning protocol. Emotional pictures were replaced by neutral ones in the control condition.

- CE1: The conditioning experiment consisted to use 2 short movies. At the end of one of then, suddenly occurred a howling degusting face (unconditioned stimuli [US]). This movie is also presented without the US (conditioned stimuli, CS). Each movie was randomly viewing 7 times. During the presentation, skin conductance was measured. Subjects had also to self-report their emotional state before and immediately after the experiment.

- CE2 : After a delay of 8 to 9h (either sleep, either waking state), participants had to respond again to the DES followed by the randomly viewing of each movie 3 times (with SCL recording). After the viewing, they responded to the DES.

In the "day" group, CC1 was performed the first day in the morning and CC2 the evening. The tomorrow morning, they performed CE1 and the evening CE2. In the "sleep" group, the first set of conditioning protocols was performed at 9:00 p.m. The second set was carried out after 1 hour. The last repetition was performed the following day, 8 hours after the first one.

e. Statistical analysis.

Our study included two main indices of emotional responses (GSR: galvanic skin responses and DES scores). As our main hypothesis was that emotional conditioning will be modulated by processing during the night, we excluded of the analysis participants for who conditioning was not effective (i.e. with no changes in DES or no GSR during the presentation of unpleasant pictures). Following these two criteria, 6 subjects in the day-group and 7 in the night group have been excluded of the statistical analysis. *This point explains that we currently include additional participants in the project to be more confident about our promising results and to publish our result on a high level international journal*

Impact of sleep on emotion regulation was assessed using repeated ANOVA measures that included two groups levels (sleep and day groups), and four periods of analysis (for DES: before the CE1, after CE1, before CE2 et after CE2; for GSR: CC1, CC2; CC3, CC4). In addition, we compared GSR for emotional and neutral movies.

qEEG indices were analyzed using repeated ANOVA measures that included two groups levels (sleep and day groups), and two levels of electrode site (left and right temporo-occipital areas).

The Hyunh-feldt correction was employed to adjust the degrees of freedom of the F ratios. For the group factor, Bonferonni correction tests were used. The significant level was set at .05 (two-tailed). *Post hoc* comparisons of pairs of means were performed with the Newman-Keuls test. The comparison of the groups in every sleep stages was realized for all the parameters in all the frequency bands.

• Results

a. Check up of the emotional induction

Electrodermal responses

First, we checked up that our emotional stimuli induced affective states. We found a significant difference between the amplitude of the emotional stimuli (US) and neutral ones ($F(1,12) = 6.89$; $p<0.05$) with no difference between day and sleep group ($F(1,12) = 0.316$; ns).

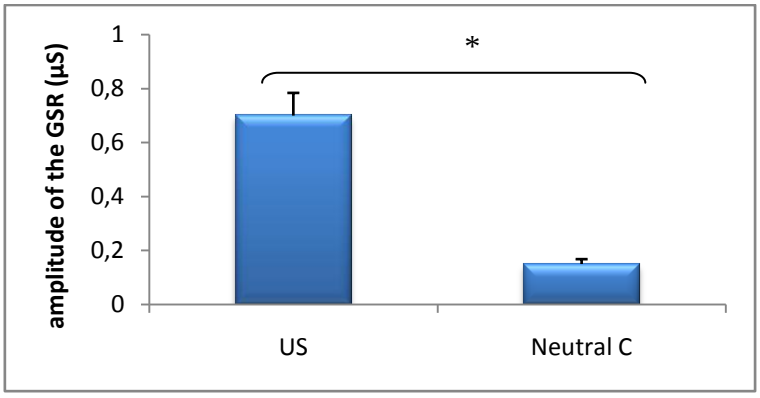
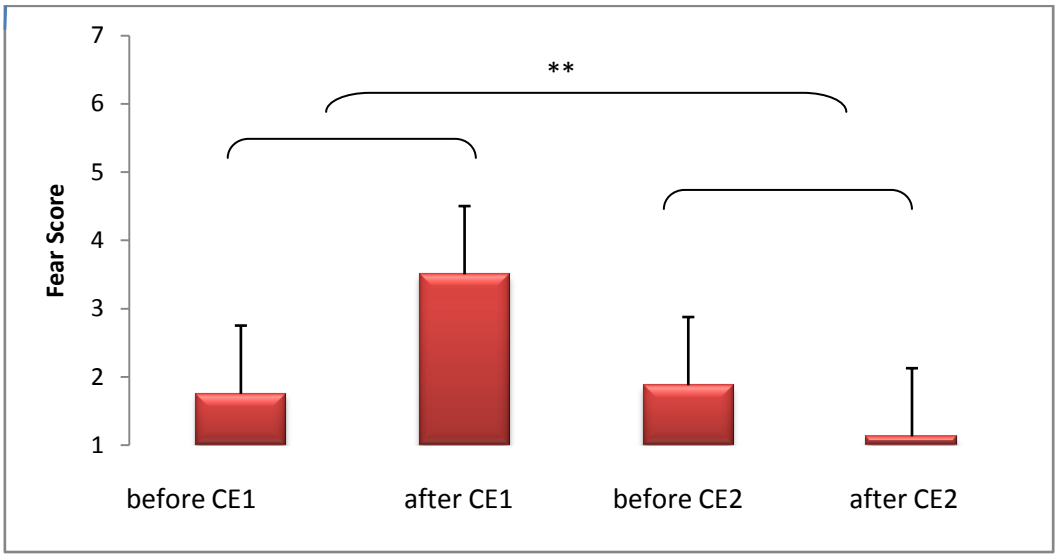


Fig. 1 : mean GSR in emotional and neutral condition for all groups

Emotional feelings

We report here results on relevant emotional scales: Fear and anxiety

Figures 2a&b show a significant interaction group * period for both anxiety and fear scales (anxiety: $F(3,36)= 27.19$; $p<0.01$; fear: $F(3,36) = 12.23$; $p<0.01$) stemmed from a significant reduction of emotional scores by sleep (anxiety : $F(1,12) = 6.65$; $p<0.05$; fear: $F(1,12) = 4.74$; $p<0.05$) whereas no difference between scores post conditioning was observed in day group (anxiety: $F(1,12) = 2.01$; ns; fear: $F(1,12) = 0.87$; ns).



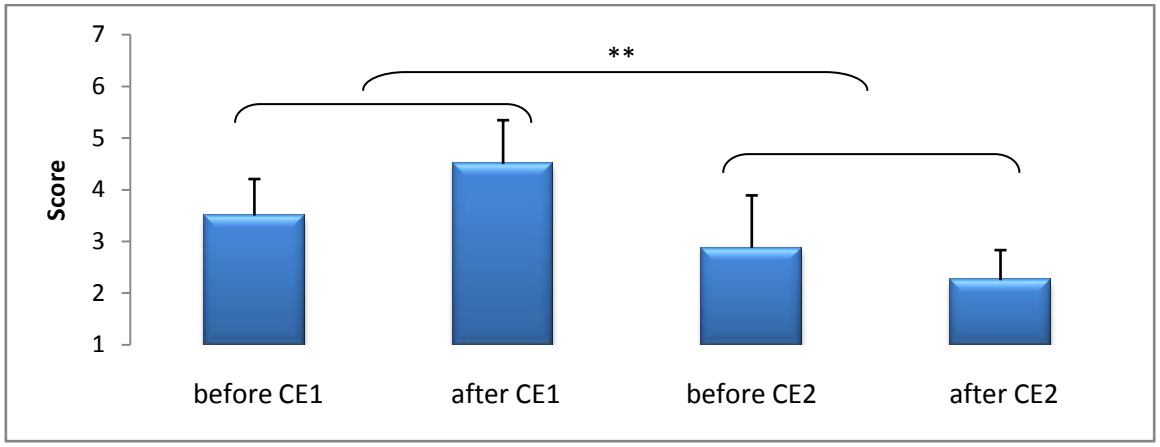


Figure 2a et 2b show differences between day group-sleep group at each period for fear scores (upper) and anxiety scores (bottom).

*** = p<0.05; ** p<0.01**

b. qualitative Sleep EEG

We assessed differences between control and emotional conditions for each parameter of global sleep and each sleep stage. Significant differences occurred between condition only for REM sleep latency and duration times. Consequently, the next stage (quantitative analysis) have been performed specifically on REM sleep

	Control night	**Emotional night**
Global measures of sleep		
SPT	460 +/- 98	510 +/- 77
TST	381 +/- 45	412 +/- 88
Sleep latency (min)	12 +/- 7	16 +/- 6.3
REM sleep latency (min)	157 +/- 67	129 +/- 58*
Sleep efficiency (%)	81 +/- 10	72 +/- 10
Awakenings	8 +/- 5	9 +/- 2
Sleep architecture (% TST)		
Stage 1 NREM	7.8 +/- 3.2	8.3 +/- 2
Stage 2 NREM	56.9 +/- 4.4	47.5 +/- 12
SWS	19.7 +/- 6.6	23.3 +/- 12.6
REM sleep	15.6 +/- 5.5	23.5 +/- 6.4*

c. quantitative Sleep EEG

During REM sleep, MF analysis revealed significant differences between groups only for the theta band. The significant interaction group x electrode site ($F(1,6) = 16.12$, $p<.01$) stemmed from a hemispheric corresponding to a higher MF at left site.

- ## Discussion

As recently described in sleep privation (Pace-schott, 2009) we observed that emotional responses to recent affective learning is reduced by sleep compared to emotional responses reported in the wake group. This result suggests that during sleep stages, processing are engaged to actively reduce emotional impact of new negative learning occurring in the previous day.

In addition, we demonstrate for the first time that brain processing sustaining emotion regulation processing during the sleep modifies both the duration of REM sleep and the neuronal activity during this stage. In particular, our result suggests that higher activity in the theta band could index emotional regulation processing during the night. By contrast EEG activity during both light and deep sleep appears to be globally unaffected by experimental conditions. In humans, theta rhythm has been repetitively associated to frontal control of cognitive process, in particular during memory encoding (see Klimesch 1996, 2008 for details). If our results are confirmed with additional subjects, we will show original findings suggesting that similar control process could occur during the REM sleep for affective information.

2. Evolution of the project

We submit our data to the main congress on emotion with the following title: Hot, P., Sabourdy C., Vercueil L. Sleep-dependent emotion regulation. ISRE2013, Berkeley, 2-3 august 2013.

We have found additional funds to increase the number of participants in both conditions. Consequently we plan to submit an article on the main results of this study in December 2013 to *Sleep* journal.

Moreover, we will stay three months in Montreal as « invited professor » in the Institute of Mental Health to discuss how to extend our study on Post traumatic disorder syndrome.

Please note that we detail in all published and oral communications that this work has been granted by BIAL foundation.