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LEARNED MATERIAL CONTENT AND ACQUISITION LEVEL MODULATE CEREBRAL REACTIVATION DURING POST-TRAINING REM SLEEP

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ABSTRACT

We have previously shown that several brain areas are activated both during sequence learning at wake and during subsequent rapid-eye-movements (REM) sleep (Maquet et al., Nature Neuroscience 3, 831-836, 2000), suggesting that REM sleep participates in the reprocessing of recent memory traces in humans. However, the nature of the reprocessed information remains an open question. Here, we show that regional cerebral reactivation during post-training REM sleep is not merely related to the acquisition of basic visuo-motor skills during prior practice of the serial reaction time task, but rather to the implicit acquisition of the probabilistic rules that defined stimulus sequences. Moreover, functional connections between the reactivated cuneus and the striatum – the latter being critical for implicit sequence learning – are reinforced during REM sleep after practice on a probabilistic rather than on a random sequence of stimuli. Our results therefore support the hypothesis that REM sleep is deeply involved in the reprocessing and optimization of the high-order information contained in the material to be learned. In addition, we show that the level of acquisition of probabilistic rules attained prior to sleep is correlated to the increase in regional cerebral blood flow during subsequent REM sleep. This suggests that post-training cerebral reactivation is modulated by the strength of the memory traces developed during the learning episode. Our data provide the first experimental evidence for a link between behavioral performance and cerebral reactivation during REM sleep.

INTRODUCTION

Sleep is believed to participate in the long-term consolidation of recent memory traces (Maquet, 2001; Smith, 2001; Stickgold et al., 2001). Although the mechanisms of memory reprocessing during sleep are not yet fully understood, the hypothesis implies that memories recently acquired during wakefulness are actively restructured and strengthened during sleep. These processes would thus promote dynamic plastic changes in neuronal populations previously engaged in memory acquisition (Maquet, 2001). Consistently with this hypothesis, several animal studies have shown that neural activity expressed during waking behavior is reinstated during subsequent sleep (e.g., Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996; Qin et al., 1997; Nadasdy et al., 1999; Louie and Wilson, 2001).

In humans, we have previously reported experience-dependent reactivation in cortical neuronal ensembles during rapid-eye-movements (REM) sleep after extended practice on a probabilistic serial reaction time (SRT) task (Maquet et al., 2000), a well-known paradigm of implicit sequence learning. In the probabilistic SRT task (Cleeremans and McClelland, 1991), subjects had to press as fast and as accurately as possible on the key that corresponds to the location of a stimulus displayed at one of six possible locations on a computer screen. Unknown to subjects, the material contained sequential structure. The sequence of successive locations visited by the stimulus over trials was probabilistically determined by an artificial grammar (Figure 1). Using positron emission tomography (PET), we showed that several brain areas activated during practice on the SRT task during wakefulness were significantly more active during subsequent REM sleep in subjects who had been previously trained on the task than in subjects without prior SRT practice. Consequently, we suggested that experience-dependent cerebral reactivation during post-training REM sleep reflected

the reprocessing of the memory traces formed during SRT practice. However, because the analyses compared subjects trained on the SRT task to subjects without any SRT practice, it was not possible to ascertain whether post-training REM sleep reactivation related to the reprocessing of elementary visuo-motor associations or of the complex sequential rules prescribed by the artificial grammar.

Here, we report a complementary study aimed to test the hypothesis that cerebral reactivation during post-training REM sleep specifically reflects the reprocessing of high-order information about the sequential structure of the material to be learned. The alternative interpretation is that experience-dependent reactivation during REM sleep is merely related to the reprocessing of the simple visuo-motor associations between stimulus location and key response. To explore these issues, we scanned a new group of subjects during sleep after practice on the same SRT task, but using a completely random sequence of stimuli. The experimental protocol was thus identical in all respects with that used for the trained group in our original study (Maquet et al., 2000), except for the absence of sequential rules. Because visuo-motor training is strictly comparable in both cases, possible differences in post-training regional cerebral blood flow (rCBF) between the subjects trained respectively to the probabilistic SRT task or to its random version could only be interpreted as reflecting specifically the reprocessing of the high-order, elaborated, sequential information after probabilistic sequence learning. Moreover, functional connections during REM sleep should be reinforced between the reactivated areas and cerebral structures involved in sequence learning for the group trained specifically on probabilistic material. Finally, we could also expect to observe that regional brain reactivation during post-training REM sleep is modulated by the level of learning achieved prior to sleep.

MATERIAL AND METHODS

Subjects: Thirteen right-handed male healthy volunteers (age range 20.5-27.0 years) participated in this complementary experiment approved by the Ethical Committee of the University of Liège. Subjects spent 2 successive nights on the scanner couch under polygraphic recording. Only subjects who showed at least 2 periods of REM sleep, 2 periods of slow wave sleep (SWS) and 2 periods of stage 2 sleep of 15 consecutive minutes each were scanned with PET during the third night after practice on the SRT task (see below). These criteria were met in 7 out of the 13 subjects. Polygraphic recordings included electroencephalogram (EEG recorded between electrode pairs C3-A2 and C4-A1), electro-oculogram (EOG) and chin-electromyogram (EMG), and were scored using international criteria (Rechtschaffen and Kales, 1968). In all 7 subjects but one, we obtained at least 2 waking, 2 stage II sleep, 2 slow wave sleep and 3 REM sleep PET scans during the third night. The 6 remaining subjects were included in the analyses described below and constituted the Random group (age range 20.5-23.5 years).

In addition, PET data from the 3 groups of our original study (Maquet et al., 2000) were re-analyzed using a random effect model (see below), thus yielding another 3 groups included in this study: Wake (n = 7; SRT practice during wakefulness), Probabilistic (n = 6; sleep after probabilistic SRT practice) and Control (n = 5; sleep without any prior practice). Behavioral and brain imaging analyses related to the high-order acquisition of sequential rules in the Probabilistic group are reported for the first time in the present communication.

SRT task: Participants faced a 17' computer screen where six permanent position markers were displayed horizontally above six spatially compatible response keys. A single SRT block consisted of 205 successive trials. On each trial, a black dot appeared 2 cm below one of the position markers, and the task consisted of pressing as fast and as accurately as possible with the right hand on the corresponding key. The next stimulus was displayed after a 200 ms response-stimulus interval.

Volunteers in Probabilistic and Random groups were exposed to two 24-block sessions between 16:00 and 20:00 PM before the experimental night with PET scanning (sessions A and B, 4920 trials each), and to one 24-block session on the following day between 16:00 and 18:00 (session C, 4920 trials). Subjects in the Wake group underwent 6 probabilistic SRT blocks during PET scanning. Subjects in the Control group (sleep without any prior practice) were not exposed to the SRT task. They remained in the laboratory between 16:00 and 20:00 PM and did not have intensive or continuous activity before sleep (Figure 2).

In the Wake and Probabilistic groups, unknown to participants, the sequential structure of the material was manipulated by generating series of stimuli based on a probabilistic finite-state grammar (Figure 1) that defined legal transitions between successive trials. To assess learning of the probabilistic rules of the grammar, there was a 15% chance, on each trial, that the stimulus generated based on the grammar (grammatical, G) was replaced by a non-grammatical (NG), random stimulus. Assuming that (implicit) response preparation is facilitated by high predictability, G stimuli should thus elicit faster responses than NG stimuli, but only if the context in which stimuli may occur has been encoded by participants. In this task, contextual sensitivity emerges through practice as a gradually increasing difference between the reaction times (RTs) elicited by G and NG stimuli occurring in specific contexts set

by at most two to three previous trials (Cleeremans and McClelland, 1991; Jimenez et al., 1996). Hereafter, we estimated, in each block, the differences between RTs elicited by G and NG stimuli in comparable contexts defined by a single previous stimulus.

To find out whether subjects in the Probabilistic group had gained any explicit knowledge about the sequential material, they were told, after completion of the last SRT block in post-sleep session C that a set of complicated rules had been used to determine the sequence of stimuli. They were then asked to perform a so-called generation task, in which they had to predict the location of the next stimulus instead of reacting to the current one. In this generation task, the material consisted of 410 grammatical trials presented over two blocks. Prediction responses were analyzed by assessing their accuracy given one element of temporal context. Chance level was determined by conducting twelve computerized simulations of continuous random prediction, with the constraint that immediate repetitions were forbidden. The simulated prediction responses were analyzed in the same manner as used for human subjects, and were then compared to participants' performance (for details on SRT and generation tasks, see Peigneux et al., 2000).

In the Random group, subjects were exposed to a random sequence of stimuli during pre-sleep sessions A and B. For comparison purposes, and even though the material does not contain sequential structure, we nevertheless categorized a posteriori each stimulus as G or as NG, as defined by consistency with the previous stimulus as prescribed by the grammar. Of course, no differences in RTs are expected between these so-called G and NG stimuli after the practice of a random sequence. Therefore, any global RT improvement in pre-sleep sessions A and B, occurring for all stimuli types through practice, should be taken to simply reflect visuo-motor skill acquisition.

In addition, to control for the possibility that subjects in the Random group could have been poor learners had they been trained on probabilistic sequential material before sleep, we asked them to perform the SRT task with the probabilistic sequence during blocks 1-20 of the post-sleep session C. If subjects in the Random group have normal learning abilities, then a gradual difference should be observed through the course of session C between RTs elicited by G and NG stimuli. Note that the change from a random sequence in pre-sleep sessions to a probabilistic sequence in the post-sleep session prevented us from analyzing overnight performance improvement and from estimating the correlation between rCBF during post-training REM sleep and subsequent performance. We therefore compared the Random and Probabilistic groups in pre- and post-sleep sessions separately.

PET data acquisition: PET data were acquired on a Siemens CTI 951 R 16/31 scanner in 3D mode. The subject's head was stabilized by a thermoplastic facemask secured to the head holder (Truscan imaging, MA), and a venous catheter inserted in a left antebrachial vein. Regional CBF was estimated during twelve 90-seconds emission scans using automated slow intravenous water ($H_2{}^{15}O$) infusion (6 mCi/222 MBq in 5 cc saline). Data were reconstructed using a Hanning filter (cutoff frequency: 0.5 cycle/pixel) and corrected for attenuation and background activity. A transmission scan was acquired to perform measured attenuation correction.

In the Wake group, subjects were scanned during SRT practice (6 scans, one SRT block each) and during quiet wakefulness at rest with the eyes closed (6 scans). Volunteers in Random, Probabilistic and Control groups were polygraphically monitored during three consecutive nights spent in the PET scanner. They were familiarized with the experimental settings and sleeping conditions during the first 2 nights. Twelve scans were performed during the third night. In all subjects, we

obtained at least 2 waking, 2 stage II sleep, 2 SWS and 3 REM sleep scans. Waking scans were obtained at rest with eyes closed in complete darkness. Sleep scans were performed when polysomnography showed steady characteristic sleep patterns (Rechtschaffen and Kales, 1968). Only data obtained during waking and REM sleep scans are discussed in the present report.

PET data processing: PET data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB (Mathworks Inc., Sherborn, MA). For each subject, all scans were realigned together, then normalized to a standard PET template and smoothed (16 mm full width at half maximum). The condition and subject (block) effects were estimated according to the general linear model at each voxel (Frackowiak et al., 1997). Realignment parameters (translations in x, y, z directions and rotations around x, y, z axes) were incorporated as nuisance variables in the design matrix (Brett et al., 1999) to account for residual movement artifacts. Unless otherwise specified, effects were computed at the random effects (RFX) level (Friston et al., 1999) to take into account within- and between-individual variability of rCBF changes during sleep. Global flow adjustment was performed by proportional scaling. The resulting set of voxel values for each contrast constituted a map of the t statistic [SPM(T)], thresholded at $p \leq 0.001$ ($T \geq 1.93$). Unless otherwise specified, statistical inferences were then obtained at the voxel level corrected for multiple comparisons in the brain volume ($p^{corr} < .05$).

First, using an RFX analysis, we looked for those brain areas in which neural reactivation during post-training REM sleep is specific to the presence of a probabilistic sequence in the training material. At the within-subject level, primary contrasts were used to estimate in each individual the main effects of (1) practice on the SRT task versus rest (in the Wake group) and of (2) REM sleep versus

wakefulness (in Random, Probabilistic and Control groups). The resulting contrast images were used in the second level (random effects) analysis, where subjects are considered as random variables. The condition [REM versus wakefulness] by Group [Probabilistic versus Random] interaction identified the brain regions that were more active during post-training REM sleep (as compared to wakefulness) in subjects having practiced a structured rather than a random sequence of stimuli in the SRT task before sleep. Next, to ensure that these areas indeed participated in SRT performance during wakefulness, we used a conjunction analysis to reveal the commonalities between this set of brain areas and the areas activated by actual practice of the SRT task in the Wake group [SRT versus Rest]. Finally, an inclusive mask (thresholded at $p < 0.05$ uncorrected) was applied to the conjunction to restrict the analysis to the brain areas that were more active during REM sleep after SRT practice than during REM sleep without any prior practice, i.e., the [Probabilistic versus Control] group by [REM versus wakefulness] interaction. Additionally, we searched for brain areas in which reactivations during post-training REM sleep might be due to the mere effect of SRT practice without a sequential structure. To do so, a conjunction analysis looked for brain areas that were both (1) activated by actual practice of the SRT task in the Wake group [SRT versus Rest] and (2) more active during REM sleep after random SRT practice than during REM sleep without any prior practice, i.e., the [Random *versus* Control] group by [REM versus wakefulness] interaction. Note that by using conjunctions at the random effects level, we implicitly assume sphericity of the error variance. The significance threshold for resulting activations was set at $p^{corr} < .05$, after correction in the brain volume.

Second, using a psychophysiological interaction analysis (Friston et al., 1997), we tested the hypothesis that if reactivated brain areas participate in the reprocessing of

probabilistic sequential information during post-training REM sleep, they should establish or reinforce functional connections with other brain regions known to be involved in high-order sequence learning during wakefulness. The effects of 3 covariates of interest were estimated according to the general linear model at each and every voxel using a fixed effect model. The 3 covariates consisted of the group effect (Probabilistic versus Random) and of the adjusted rCBF of two reference areas shown to be reactivated during post-training REM sleep depending on the presence of probabilistic rules in the training material (i.e., the left and right cuneus, coordinates -30 -70 16 and 32 –68 12 mm; see Results section). The interaction analysis [Group by adjusted CBF in reference areas] identified the brain areas that were functionally more related to the activity in the cuneus during post-training REM sleep in subjects having practiced a structured rather than a random sequence. Neuroanatomical hypotheses were based on an independent study (Peigneux et al., 2000) showing significant involvement of the striatum (caudate nucleus and putamen) and of the middle (BA 10) and inferior frontal gyri (BA 44, 45, 46, 47) in high-order implicit learning during the practice of the probabilistic SRT task. Results in these regions were considered significant at $p^{SVC} < .05$, after correction in a small spherical volume (radius 20 mm). Significance level was otherwise set at $p^{corr} < .05$.

Finally, using an RFX model, we investigated the relationship between the strength of the memory traces acquired prior to sleep and subsequent modifications of regional cerebral activity during REM sleep in sequence-related cerebral areas. At the within-subject level in the Probabilistic group, primary contrasts individually estimated the main effect of REM sleep versus wakefulness. At the between-subject level, this third analysis looked for the regression of pre-sleep high-order learning stabilized performance (mean RT [NG–G] differences in session B) on post-training CBF

variations during REM sleep (versus wakefulness). Statistical inferences were obtained at the voxel level in the small volume of the brain areas identified in the first analysis. To test if basic visuo-motor skill acquisition also contributes to subsequent rCBF changes during REM sleep, a fourth analysis similarly looked for the regression of pre-sleep low-order learning improvement (mean global RT in session B subtracted from the mean global RT in the 4 first blocks in session A, irrespective of item grammaticality) on post-training CBF variations during REM sleep (versus wakefulness) in both Probabilistic and Random groups. Statistical inferences were obtained at the voxel level in the small volume of the brain areas identified in the Maquet et al. (2000) analysis (i.e. Wake [SRT vs. Rest] in conjunction with the [Probabilistic vs. Control] by [REM vs. wakefulness] interaction).

RESULTS

Behavioral data

Incorrect responses, values outside of 2 standard deviations from the mean and the first five trials of each block were discarded from the analyses. Figure 3 shows the mean RTs elicited by G and NG stimuli across the 72 blocks practiced during pre-sleep (A, B) and post-sleep (C) sessions. Mean (standard deviation) session RTs elicited by G and NG stimuli were respectively 545 (± 57) vs. 562 (± 60) ms (session A), 507 (± 33) vs. 533 (± 38) ms (B), 425 (± 24) vs. 447 (± 30) ms (C) in the Probabilistic group, and 530 (± 45) vs. 540 (± 53) ms (A), 504 (± 29) vs. 501 (± 32) ms (B), 453 (± 31) vs. 466 (± 41) ms (C) in the Random group. A four-way analysis of variance (ANOVA) yielded a significant interaction effect between group [Probabilistic vs. Random], grammaticality [G vs. NG], session [A vs. B vs. C] and blocks [24 repetitions] factors, $F(46,460) = 1.68$, $p < .005$.

With respect to elementary visuo-motor skill acquisition, the interaction effect between group [Probabilistic vs. Random], session [A vs. B vs. C] and blocks [24 repetitions] factors irrespective of grammaticality was non significant ($p > .98$), suggesting that global reaction times changed in a similar way in subjects trained on probabilistic and random SRT material. Significant Session and Block effects ($ps < .005$) confirmed global RT improvement through practice. Overnight effect (B vs. C) was significant in both groups ($ps < .05$) for simple visuo-motor acquisition.

With respect to high-order probabilistic rules learning, a three-way analysis of variance yielded a significant interaction effect ($F(1,10) = 6.08$, $p < .05$) between Group [Probabilistic vs. Random], Grammaticality [G vs. NG] and pre-sleep Session [A vs. B] factors. Post-hoc analyses showed that the effect of grammaticality in the Probabilistic group was significant during pre-sleep session B ($p < .05$) but not during session A ($p = .1$). This suggests, consistently with previous results, that learning of the sequential constraints set by the grammar is a gradual process (Cleeremans and McClelland, 1991; Jimenez et al., 1996). In the Random group, as expected given the absence of temporal context during learning, pseudo-grammaticality effects were not significant in pre-sleep sessions A and B ($ps > .25$). During post-sleep session C, in which both groups were exposed to the probabilistic sequence, the effect of grammaticality was significant in the Probabilistic group ($p < .05$). In the Random group, the effect of grammaticality turned significant in blocks 11 to 20 of session C ($p < .05$). Demonstration of increasing sensitivity to sequential regularities when exposed to the probabilistic sequence during post-sleep session C in the Random group rules out the possibility that differences between Probabilistic and Random groups in post-training cerebral reactivation during REM sleep could be due to differences in learning abilities.

In the Probabilistic group, RTs elicited by G stimuli were faster than RTs elicited by NG stimuli, more in the post-sleep than the pre-sleep session, but the overnight effect did not reach significance. However, we found that coefficients of variation significantly decreased overnight for G stimuli only (0.23 vs. 0.21, $p < .01$), which may suggest a qualitative reorganization of the cognitive processes (Segalowitz and Segalowitz, 1993) engaged in sequential performance during post-sleep SRT session.

In the generation task, the interaction effect between condition [Probabilistic group vs. Computerized simulation of random prediction], and grammaticality [G vs. NG] factors was non significant, $F(1,34) = 1.18$, $p < .29$, thus suggesting that subjects' performance on the explicit generation task did not differ from chance and that learning was therefore essentially implicit.

Brain imaging

Preliminary analyses conducted on primary contrasts irrespective of prior experience revealed common rCBF increase during REM sleep (versus wakefulness) in Random, Probabilistic and Control groups in a set of brain areas previously reported to be involved in REM sleep generation (Maquet et al., 1996; Braun et al., 1998), including brainstem and thalamus, limbic (amygdaloid complexes, hippocampal formation, anterior cingulate cortex), pre- and post-central, and temporo-occipital areas ($p < .001$ uncorrected).

The first analysis showed that rCBF in the left [standard stereotactic coordinates -30 –70 16 mm] and right [32 –68 12 mm] cuneus both increased during SRT practice (Wake group) and increased more during post-training REM sleep in Probabilistic than Random group ($p^{corr} < .05$; Figure 4a). The reactivation of these areas during post-training REM sleep is specifically related to the presence of sequential organization in the material to be learned. At a lower statistical threshold ($p < .001$,

uncorrected), additional reactivations were found in the premotor cortex [-24 0 52 mm] and mesencephalon [-2 –36 –18 mm]. Peak coordinates in these areas are less than 12 mm from previously published brain areas that activated more during REM sleep in the Probabilistic than in the Control group and also during SRT practice in the Wake group (see Table 6 in Maquet et al., 2000). Slight shifts of the location of peak activation sites were expected due to the transition from a fixed-effect analysis in the Maquet et al. (2000) study to a random-effect analysis in the present study. Also, activity in the thalamus increased more during REM sleep after the practice of the probabilistic than the random sequence [-16 –34 –4; $p < .001$], but was not conjointly activated during the SRT task. The additional analysis failed to reveal brain areas in which rCBF both increased during SRT practice in the Wake group and increased more during post-training REM sleep in Random than Control group ($p^{corr} > .17$), suggesting that random practice on the SRT task is not likely to elicit significant rCBF modifications in the post-training REM sleep.

Second, the psychophysiological interaction analysis showed that rCBF in the left and right cuneus (at stereotactic coordinates cited above) correlated significantly more with CBF during post-training REM sleep in Probabilistic than Random group in the left [18 -12 20 mm] and right [-16 -6 24 mm] caudate nucleus, respectively ($p^{SVC} < .05$; Figure 4b).

Third, the regression analysis revealed a significant correlation (Figure 5) between pre-sleep high-order learning performance level during session B [mean rT[NG-G]) and subsequent REM sleep CBF changes (versus wakefulness) in the anterior portion of the cuneus, i.e. the depth of the right parieto-occipital fissure (26 -70 24 mm in standard anatomical space, coefficient of correlation $r = 0.87$), and in the cingulate sulcus (22 -26 32 mm, $r = 0.94$; $ps < .001$, uncorrected). The correlation between CBF

and pre-sleep performance in the Probabilistic group reflects a modulatory role of the level of prior learning on CBF activity during the subsequent REM sleep. Finally, the fourth analysis failed to reveal any significant correlation between pre-sleep low-order learning performance improvement (mean RT) and rCBF variations during REM sleep in Probabilistic and Random groups.

DISCUSSION

We have shown that during post-training REM sleep, rCBF in left and right cuneus increased more in subjects who had been previously trained on a probabilistic sequence of stimuli rather than on a random one. Importantly, both groups had been exposed to identical SRT tasks that differed only in the underlying sequential structure of the stimuli prior to sleep. Our results therefore suggest that reactivation of neural activity in the cuneus during post-training REM sleep is not merely due to the acquisition of basic visuo-motor skills. Rather, it reflects the reprocessing of elaborated information about the sequential contingencies contained in the learned material. This interpretation is further corroborated by the finding that rCBF during post-training REM sleep is modulated by the level of high-order, but not low-order, learning attained prior to sleep. Therefore, these results suggest that neural activity during REM sleep in brain areas already engaged in the learning process during wakefulness could be modulated both by the *sequential structure* of the practiced material and by the *amount of high-order learning* achieved prior to sleep.

Methodological note: Functional connectivity and the integrative brain during post-training sleep

Brain function relies on both functional segregation and integration (Friston, 2002). Segregation indicates that specific brain areas participate in the treatment of certain

types of information, whereas integration emphasizes that cerebral functions emerge from cooperative activity between various brain areas. Our first analysis revealed more experience-dependent activity in the bilateral cuneus during REM sleep after probabilistic than random SRT training. The following psychophysiological interaction showed that rCBF activity in the cuneus during post-training REM sleep correlates tightly more to caudate nucleus activity in the Probabilistic than in the Random group. The two analyses fundamentally differ. The former indicates that segregated brain areas are specifically engaged in the post-processing of sequential information, because cuneus activity is both present during SRT practice and higher during REM sleep (versus Wake) in the Probabilistic than in the Random group. On the other hand, the psychophysiological interaction emphasizes cortical integration (Friston *et al.*, 1997) and tests for a difference in the regression slope of activity between remote areas over time under different levels of a psychological factor. These regression slopes can be thought of as a measure of coupling in the sense that they reflect the change in one area for a unit change in another area. Therefore, the analysis indicates that coupling of cuneus and caudate nucleus activity during post-training REM sleep is increased by the presence of a sequential structure in the SRT task, which do not entail that mean caudate activity is higher in the Probabilistic than in the Random group. Moreover, our current knowledge of the cerebral correlates of sleep mechanisms is still fragmentary. It is likely that the amplitude of the reactivation in cortical and subcortical areas is modulated at the regional level by sleep mechanisms. This may explain why caudate nucleus activity did not show off in the same (segregation) analysis that showed cuneus reactivation. Further studies will be needed to elucidate regional differences in post-training reactivation during sleep between areas engaged in training during wakefulness.

Does the sleeping brain like structure? Importance of the structure of the learned material for memory processing

The processing of recent memories during post-training sleep does not seem to be initiated unless the material to be learned is structured. If the material does not contain any structure, as is the case in the random SRT task, no significant post-training REM sleep reactivation was observed. Although we cannot preclude the hypothesis that absence of significant reactivations during post-training REM sleep in the Random group might be due to limited sensitivity of the $H_2{}^{15}O$ PET technique, the results are consistent with previous experiments. At the behavioral level, increase in REM sleep duration is observed in rats after aversive conditioning in which a tone is paired with a footshock, but not after pseudo-conditioning in which the tone and the footshock are given unpaired (Bramham et al., 1994). Using a similar procedure at the systems level, tone-evoked responses are obtained in the medial geniculate nucleus (Hennevin et al., 1993) and in the hippocampus (Maho et al., 1991) during REM sleep after a conditioning procedure initiated at wake, but not after pseudo-conditioning. Likewise in humans, REM sleep percentage increases after learning textbook passages, but only when they are meaningful (Verschoor and Holdstock, 1984). A similar situation involves material so complex that its underlying structure cannot be extracted through practice. For instance, post-training modifications of the sleep architecture (in this case, increases of Stage 2 sleep duration) have been reported in humans after virtual maze exploration only when the complexity of the maze allowed subjects to learn their way and form a cognitive map (Meier-Koll et al., 1999).

Does the level of learning modulate regional brain activity during post-training REM sleep?

The present data further indicate that the level of prior learning influences the activity in the cuneus during subsequent sleep. In both cerebral hemispheres, the location of the activation peak is less than 8 mm apart from the activation of the cuneus revealed by the first analysis. This result is remarkable, given that the design matrices of these two analyses were completely different. At the behavioral level, animal studies have already shown that an increase in the amount of post-training REM sleep only occurs when a sufficient level of learning has been reached (Dujardin et al., 1990; Hennevin et al., 1995; Smith, 1995). Accordingly in humans, post-training modifications of sleep parameters are also observed after conditioning in babies, but only when the stimulus-response association had been successfully learned (Paul and Dittrichova, 1975). At the systems level, experience-dependent replay of neuronal activity during REM sleep has been already demonstrated in animals (Poe et al., 2000; Louie and Wilson, 2001), but not yet proven behaviorally relevant since the link between the performance and the ensuing neural pattern during sleep has never been demonstrated (Peigneux et al., 2001). Here, we provide the first evidence that the strength of rCBF reactivation during post-training REM sleep depends on prior learning level in humans. The demonstration that neural activity that occurs during REM sleep is modulated by the level of performance achieved prior to sleep strongly supports the hypothesis that sleep is actively involved in the processing of recent memory traces. The demonstration that extended sleep deprivation prevents improvement in high-order sequence learning (Cajochen et al., 2002) gives further support to this hypothesis.

Functional relationships between REM sleep and the processing of implicit memories

The respective role of non-REM and REM sleep in memory consolidation is still debated (Peigneux et al., 2001; Smith, 2001). The dual-process hypothesis proposes

that REM sleep facilitates consolidation of non-declarative memories (Smith, 1995; Plihal and Born, 1997, 1999; Smith, 2001) whereas non-REM sleep is more specifically involved in the processing of declarative memories (Plihal and Born, 1997, 1999).

Learning the probabilistic SRT task is an implicit process. Behavioral data indicate that subjects show greater sensitivity to the sequential structure of the material during the indirect (SRT) task than during the direct (generation) task. According to Merikle and Reingold (1991), these results suggest that most of the knowledge acquired after extended practice (up to 14760 items) with the probabilistic SRT task was unconscious. At the systems level, we have found that the cuneus establishes or reinforces functional connections with the caudate nucleus during REM sleep following probabilistic SRT practice. The cuneus, which participates in the processing of the probabilistic sequence both during SRT practice and post-training REM sleep, has been previously shown to be activated during sequential information processing at wake (Schubotz and von Cramon, 2001). However, it is not commonly seen as a critical component of sequence learning. In contrast, the striatum is known to participate in implicit sequence learning (Grafton et al., 1995; Rauch et al., 1995; Rauch et al., 1997) and specifically in the encoding of the temporal context set by the previous stimulus in the probabilistic SRT task (Peigneux et al., 2000). The finding that the strength of the functional connections between cuneus and striatum is increased during post-training REM sleep suggests the involvement of the basal ganglia in the off-line reprocessing of implicitly acquired high-order sequential information. Our results are therefore consistent with the hypothesis that REM sleep facilitates consolidation of non-declarative memories. However, experience-dependent reactivation of neuronal ensembles during non REM sleep after the

acquisition of declarative memories should be demonstrated to further validate this dual-process hypothesis.

Finally, alternative interpretations suggest that the consolidation of memory traces during post-training sleep would require the succession of non-REM and REM sleep episodes (Giuditta et al., 1995; Gais et al., 2000; Stickgold et al., 2000). The present data can neither confirm or infirm these hypotheses. Future research is needed to better characterize the functional importance of non REM sleep, REM sleep and their dynamic relationships in the consolidation of memory during sleep.

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FIGURES LEGEND

Figure 1

Probabilistic finite-state grammar used to define legal transitions between successive trials, from Jimenez et al. (1996). Each letter corresponds to a stimulus location on the screen. At each node (#), one of the possible arrows (→) is randomly selected, and the corresponding label is collected and added to the probabilistic sequence of grammatical (G) stimuli.

Figure 2

Experimental design followed for subjects in the Probabilistic (P), Random (R), and Control (C) groups.

Figure 3

Average reaction times (and standard errors) per block for grammatical (G; red lines) and non-grammatical (NG; blue lines) stimuli during pre- and post-sleep SRT sessions in Probabilistic (top row) and Random (bottom row) groups. Subjects in the Random Group were exposed to a random sequence of stimuli in pre-sleep SRT sessions A and B, and to the probabilistic sequence in blocks 1-20 of post-sleep SRT session C. Note that no differences were expected between pseudo G and NG stimuli (categorized a posteriori) in pre-sleep sessions in the Random group. Each session consisted of 24 blocks of 205 successive stimuli each.

Figure 4

(a) Sequence-dependent regional cerebral reactivations during post-training REM sleep. Statistical parametric maps of the bilateral cuneus, that both activated during SRT practice (versus rest) and activated more during REM sleep (versus wakefulness; W) in Probabilistic than Random group, superimposed on the coronal section of a

subject's normalized MRI at 68 and 70 mm behind the anterior commissure. Grey bars displayed on the left side of the images illustrates the subtraction (top) and interaction (bottom) contrasts entered in the SPM99 conjunction analysis. Activations are displayed at $p < .001$, uncorrected.

(b) Sequence-dependent increase of functional connectivity during post-training REM sleep. Plot of the regression of centered rCBF in the right cuneus (32 –68 12 mm) and right caudate nucleus (18 –12 20 mm) during post-training REM sleep in subjects trained to the probabilistic SRT task (red circles) and subjects trained to the random SRT task (blue crosses). A similar regression is observed between cuneus and caudate nucleus in the left hemisphere. The inset on the right side show the activation of the right caudate nucleus (displayed at $p < .001$, uncorrected), superimposed on the transverse section of a subject's normalized MRI.

Figure 5

Learning level attained prior to sleep modulates regional reactivation during REM sleep. Regression of pre-sleep high-order performance (mean rT elicited by NG items *minus* mean rT elicited by G items on session B) on post-training REM sleep rCBF in the right cuneus ($r = 0.87$, coordinates 26, –70, 24 mm in standard anatomical space), in Probabilistic SRT trained subjects.

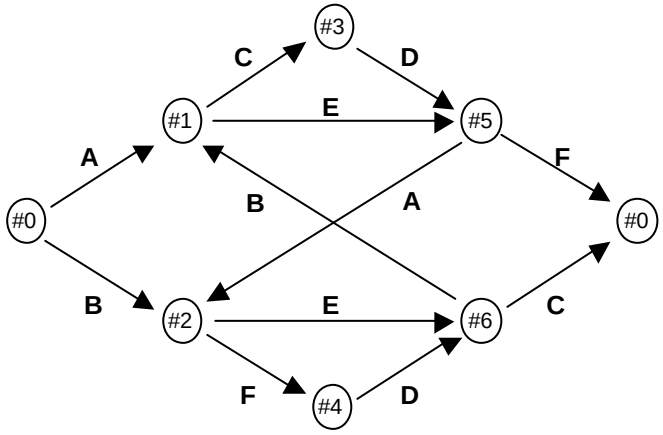

Figure 1 - Peigneux et al.

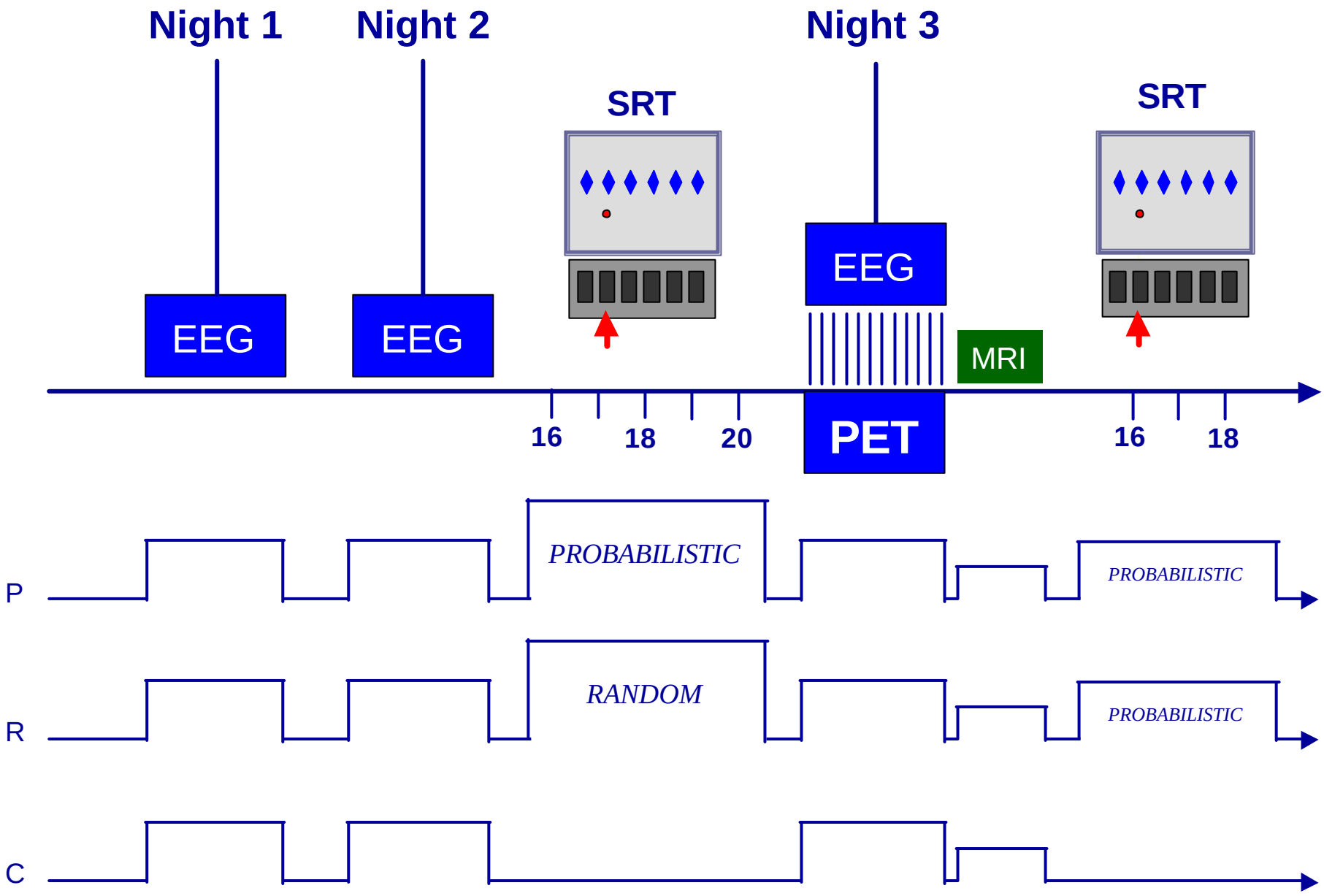

Figure 2 - Peigneuxet al.

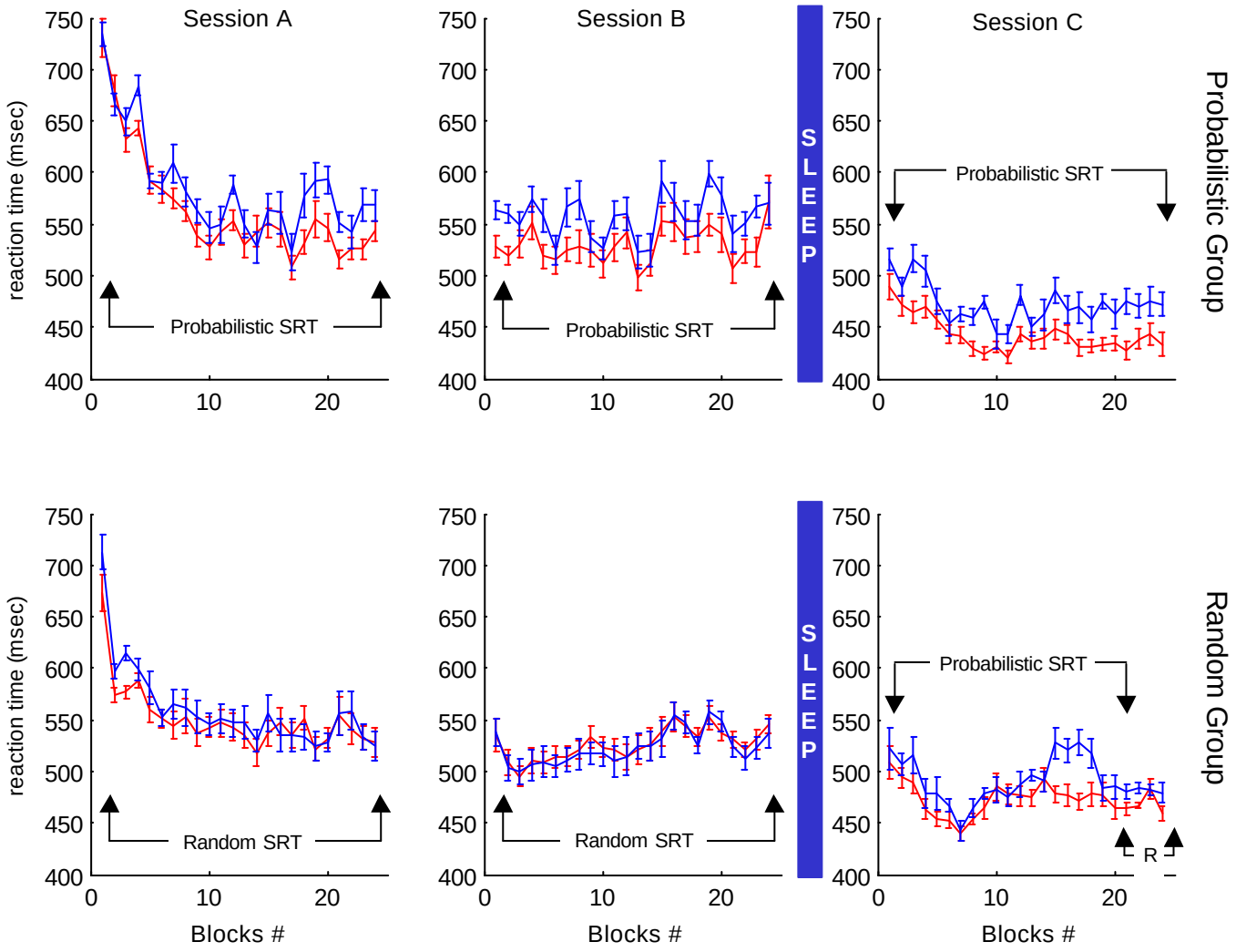

Figure 3 - Peigneux et al.

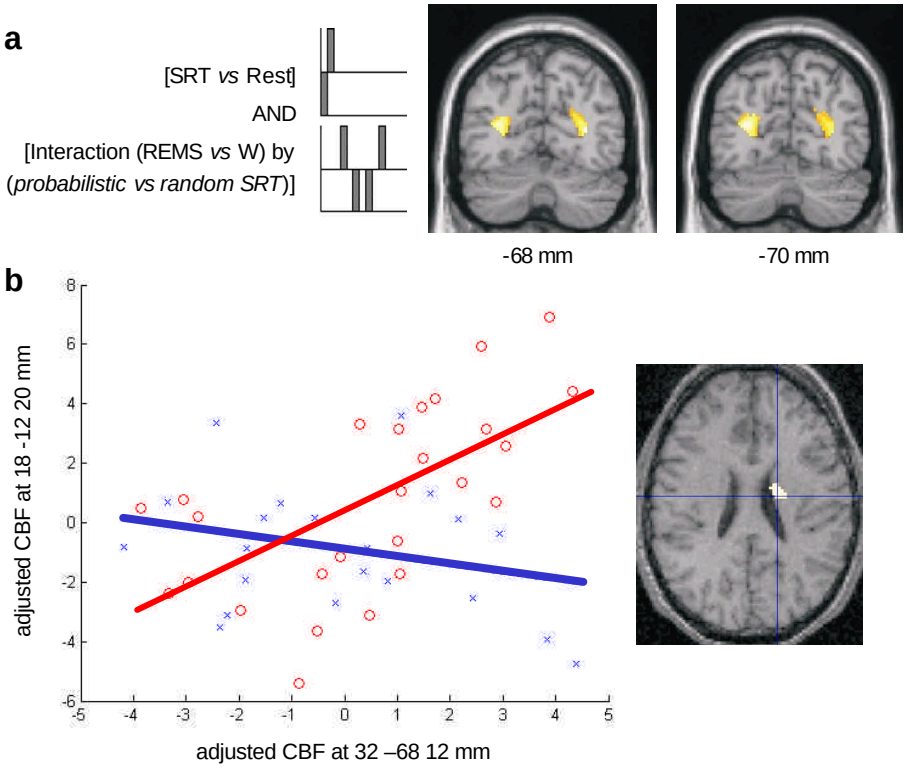

Figure 4 - Peigneux et al.

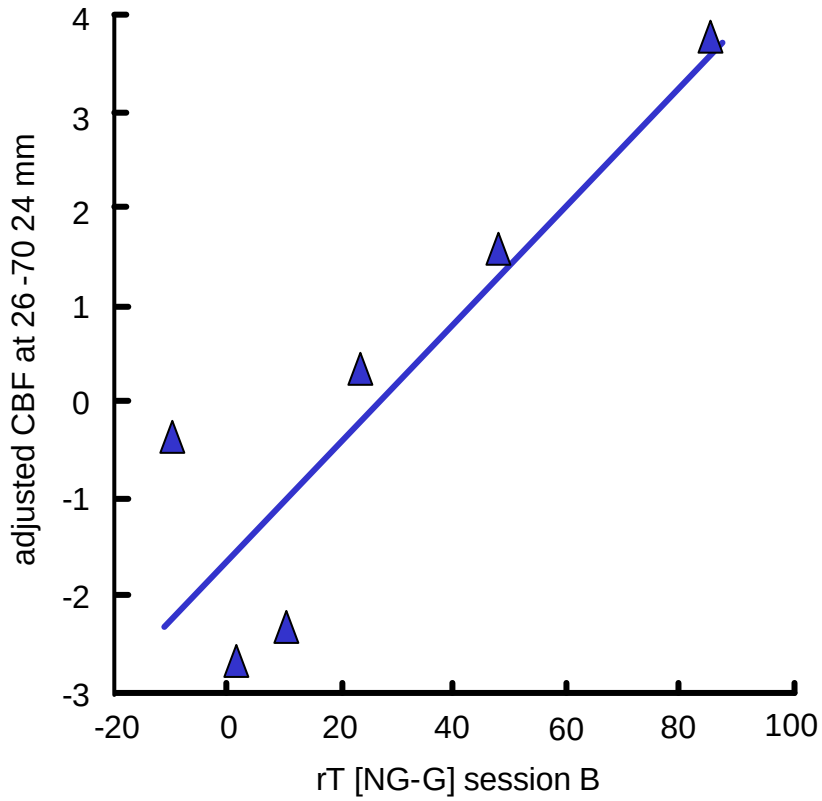

Figure 5 - Peigneux et al.