Manuscript title: Does sleep benefit memory consolidation in children with focal epilepsy?

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Abbreviations: REM=rapid eye movement sleep, NREM=non-rapid eye movement sleep, 2D=two-dimensional, IED=interictal epileptiform discharges, CSWS=continuous spike waves during sleep, SWI=spike wave index

Summary

Objective: Children with epilepsy have high rates of both cognitive impairment and sleep disruption. It is thus assumed that sleep-dependent memory consolidation is vulnerable to ongoing epileptic activity, but direct evidence of this is limited.

Methods: We performed a within-subject comparison of memory retention across intervals of wake or overnight sleep. Healthy children (n=21, 6-16 years, 12 females) and children with focal epilepsy (n=22, 6-16 years, 9 females) performed verbal and visuospatial memory tasks under each condition. Sleep was assessed with EEG polysomnography during the overnight interval. Interictal discharges were quantified manually.

Results: Memory retention was greater in the sleep condition in both the verbal [F(1,39)=10.8, p=0.002, Cohen's d=0.67] and the visuospatial [F(1,36)=4.23, p=0.05, Cohen's d=0.40] tasks, with no significant interaction of group by condition in either task. Across the total sample, gain in memory retention with sleep in the verbal task correlated with duration of slow wave sleep (r=0.4, p=0.01). In patients, sleep-dependent memory consolidation was negatively correlated with interictal discharge rate in both the verbal (Rho=-0.49, p=0.04) and visuospatial (Rho=-0.45, p=0.08) tasks. On post-hoc analysis, a longer history of epilepsy (r=0.53, p=0.01) and a temporal [t[10]=1.8, p=0.1, Cohen's d=0.86] rather than an extra-temporal seizure focus [t(10)=0.8, p=0.4, Cohen's d=0.30] was associated with greater contribution of sleep to verbal memory retention .

Significance: We have demonstrated that memory consolidation in children with focal epilepsy benefits from sleep, showing the same correlation with slow wave sleep as in healthy children, but an inverse relationship with the interictal discharge load during sleep. This mechanism appears to be increasingly recruited with longer duration of illness, indicating a resilient homeostatic function which may be harnessed to aid learning.

Key points

- Sleep in children who have focal epilepsy with structural or presumed structural etiology enhances memory consolidation to the same degree as in healthy children

- This mechanism is resilient to both chronic seizures and acute sleep disruption, though impaired by frequent nocturnal discharges

- Harnessing this ability may enhance learning in children with epilepsy

Introduction

Cognitive impairment is arguably the most important co-morbidity in childhood epilepsy, with a prevalence of 25-40%[1,2] and a major impact on long term outcome[3,4]. It has been proposed that sleep disruption and epileptic activity during sleep can account for a significant proportion of learning impairments in children with epilepsy[5,6], but beyond the rare syndrome of continuous spike waves during sleep (CSWS), direct evidence of this is limited. To advance this argument, we performed a controlled investigation of sleep and memory in children with focal epilepsy with a structural or presumed structural etiology - a group in whom the substrate of sleep and memory outside the epileptogenic site may be presumed intact[7].

Slow wave sleep is named for the characteristic widespread, high amplitude delta waves seen on EEG, representing synchronised "up" and "down" states across large populations of cortical neurons[8]. Data from healthy individuals studied over the past decade have shown that memory consolidation occurs during sleep, in both adults[9] and school-aged children[10, 11]. This is particularly true of declarative memory - that for facts and events. Sleep slow oscillations are postulated to facilitate memory consolidation through two mechanisms: active systems consolidation[9] and synaptic homeostasis[12].

Briefly, the former refers to the reactivation of hippocampal neuronal assemblies, representing transient storage of the day's learning, in a temporal framework driven by cortical slow oscillations. This timed replay ensures that neuronal firing occurs when conditions for long-term potentiation are favourable, so that new memories can be written into long term storage in the cortex[13]. The latter putative mechanism arises from the concept of sleep homeostasis[14]. Environmental stimulation

during the day leads to an overall increase in synaptic strength. During sleep, the repeated "on"/"off" firing which comprises the slow oscillations leads to global synaptic downregulation over the course of the night, until only the strongest connections - those potentiated the most during wake or best integrated with existing memories - survive[12].

Sleep in children with epilepsy is often disturbed[15,16], implying that the impairment of this process may contribute to the observed cognitive difficulties. Data from recent pilot studies[17-18] utilising memory tasks performed with concurrent EEG polysomnography suggest that sleep-related memory consolidation may be impaired in children with epilepsy. However these have lacked either a baseline condition[17, 18]or a healthy control group[19] for comparison and thus cannot isolate the contribution of sleep to memory consolidation in this population.

Here we compared a group of children with focal epilepsies of structural or presumed structural etiology to an age-matched group of healthy children. We examined the gain in items recalled with sleep and its association with sleep parameters using a within-subject comparison of memory retention over intervals with and without sleep. Specifically, we hypothesized that the children with epilepsy would show impaired memory consolidation with sleep, and that impairment would correlate i) inversely with the amount of slow wave sleep and ii) positively with the amount of interictal discharges in sleep.

Materials and methods

Participants

Data were collected on 22 children with focal epilepsy (6-16 years) and 21 healthy children (6-16 years) recruited prospectively to take part in this study. Participants had no prior diagnosis of primary sleep disorders.

Over a period of 18 months, 40 consecutive inpatients at the EEG video-telemetry unit at Great Ormond Street Hospital (GOSH) were approached, provided they met the following criteria: 1)firm diagnosis of medication resistant focal epilepsy with a structural or presumed structural etiology, 2)attendance at mainstream school (as a proxy for sufficient cognitive ability to complete the experimental tasks) and 3)planned hospital admission for at least 4 nights. Twelve declined to participate. Of the 28 who consented, three were discharged home before they could complete the study protocol, one withdrew, and two were unavailable to perform the tasks during the time window for testing. Additionally, one participant was found not to have epilepsy, and was excluded from the analysis. No apnoeas or desaturations were detected in patients apart from those associated with seizures.

Healthy children were recruited by advertisement directed at staff working at Young Epilepsy, a charity that supports children and young people with epilepsy in the United Kingdom. Additionally, healthy school-aged siblings of patient participants were invited to participate. 23 healthy children consented to take part in the study; one did not complete the study protocol. One child showed evidence of sleep disordered breathing on polysomnography and was excluded from the analysis. Only one control participant was the sibling of a patient.

Standard protocol approvals and patient consents

The study was approved by the local research ethics committee. Written informed consent for data collection was obtained from a parent for each patient.

Experimental design

We performed a within-subject comparison of memory retention across similar length intervals with or without sleep (Fig. 1). Each participant was tested on two occasions - once under each of the 'sleep' and 'wake' conditions. For each condition, participants performed parallel versions of a verbal and a visuospatial memory task. The order of task versions and order of conditions were randomised separately, using a block design for each. For each participant, the sleep and wake conditions were separated by at least 24 hours.

For the overnight ("sleep") condition, material was learned in the early evening (between 5:00 and 6:00pm), after preparation for polysomnographic recording. Participants went to bed at their habitual time, apart from patients who underwent sleep restriction for diagnostic purposes (n=6), who stayed up 2 to 3 hours later than usual. The next morning, participants were awakened at their usual time. Testing took place an hour later. The interval between learning and testing was approximately 15 hours (15.3+/-1.2). For the daytime ("wake") condition, learning took place in the morning an hour after awakening, and testing in the evening, at an approximately 8 hour (8.4+/-1.1) interval. Parents kept a record of the child's activities through the day.

Memory tasks

The memory tasks were adapted from Wilhelm and Diekelmann[10]. Verbal declarative memory was

tested using a word-pair associate learning task while visuospatial declarative memory was assessed using a two-dimensional (2D) object location task, similar in concept to the card game commonly known as 'Pairs' (see Supplementary Material for details).

At each session, the 2D object location task was presented before the word-pair associate task. For both tasks, the learning phase was as follows: 1)all the items were presented to the participant in cue-target pairs 2)the participant was asked to respond with the target object position or word when presented with each cue 3)learning trials were repeated until the participant reached a criterion score - at least 40% correct for the 2D location task and 60% correct for the word pairs, or until the participant refused further trials, whichever occurred sooner. In the testing phase of each task, the participant was given a single attempt to respond to each cue with its target, and correct responses totalled to give the test score.

The criterion score was then subtracted from the test score to obtain a 'memory retention score', representing the difference between delayed and immediate recall. Each participant thus obtained two memory retention scores for each task - one from performing the task under the "sleep" condition, and the other under the "wake" condition. The difference between the sleep and wake condition scores for each task yielded a quantitative measure of the contribution of sleep to memory consolidation ("sleep benefit") for that task, in that participant.

EEG/Polysomnography

Sleep was recorded with the Xltek Trex (Natus, USA) system, using 8 EEG electrodes (F3, F4, C3, C4, O1, O2, A1, A2) positioned according to the international 10-20 system in control subjects, and 27 EEG electrodes positioned according to the international 10-10 system in patients. In addition,

we recorded electrocardiogram, surface chin electromyogram, chest and abdominal movements and pulse oximetry in all participants. Eye movements were detected using standard electro-oculogram derivations[20] in controls, while electrodes F9 and F10 were utilised for this purpose in patients.

Sleep scoring

Sleep recordings from healthy participants, and from patients who did not have seizures during the recording were scored visually according to standard criteria[20] using Sleepworks software (Natus, USA). Sleep scored as 'N3' is equivalent to slow wave sleep.[20] For recordings containing seizures (n=5), conventional sleep stages were scored by standard criteria, but epochs were scored as "seizure" if they contained ictal discharges, >50% pre-ictal build-up or >50% post-ictal slowing. "Seizure" epochs were not included in the Total Sleep Time. For all patient recordings, we added custom bipolar channels to the standard sleep scoring montage, in order to highlight focal epileptiform activity and distinguish this from sleep phenomena.

Quantification of interictal discharges

Interictal epileptiform discharges (IEDs) included sharp waves, spikes, spike-and-slow wave complexes and polyspike-and-slow wave complexes, as defined by the International Federation of Societies for Electroencephalography and Clinical Neurophysiology[21]. The identified IED morphologies for each patient were verified with a Consultant Neurophysiologist (RP, SB).

The count was recorded as the number of IEDs per minute, calculated by dividing the total number of IEDs by the duration of sleep time over which they were counted. Discharges were marked manually for the first two sleep cycles, to include at least one period of REM. There is evidence from paediatric patients with focal epilepsies that the IED rate does not differ significantly between

sleep cycles across the night[22]. For the two patients with more than 10 discharges in most 10-second epochs, 100 IEDs were counted for each sleep stage in each sleep cycle and the result divided by the duration of EEG reviewed for each stage. For all other patients, the record was parsed manually for the whole of the first two sleep cycles. For analysis, we expressed the discharge load for each patient as the number of discharges per 100 seconds, to facilitate comparison with existing studies in the literature.

Intelligence scores and sleep habits

Full scale IQ scores on the Wecshler Intelligence Scale for Children version 4 (Pearson, USA) for the patients were extracted from recent neuropsychological reports - these were available for 21 of the 22 patients. Control subjects underwent IQ testing using the Wechsler Abbreviated Scale of Intelligence (Pearson, USA) during their participation in the study. For each participant, the Children's Sleep Habits Questionnaire (CSHQ)[23] was completed by a parent.

Statistical analyses

A repeated measures ANOVA was used to investigate the effect of sleep on memory retention for each task, with Condition (sleep or wake) as the within-subject factor and Group (patient or control) as the between-subjects factor. Paired sample t-tests were used for post-hoc comparisons between the sleep and wake conditions for controls and patients respectively. The same statistical tests were employed for patient subgroup analyses.

Pearson's r correlation coefficient was used to investigate the association of gain in performance with sleep between the two memory tasks, and the relation between sleep architecture parameters and gain in memory performance with sleep. Independent two-group t-tests (Welch) were used to

compare sleep architecture parameters.

The association between memory scores and clinical factors in the patient group was examined with Pearson's r correlation coefficient. The relationship between memory consolidation and interictal discharge count was investigated with Spearman's Rho, because the distribution of counts remained positively skewed after log transformation. Log transformation was performed using the formula $y=\log10(x+1)$, due to the number of patients (n=4) with zero discharges.

Power calculation

We anticipated an effect size on memory score (of group and condition) at least equal to the standard deviation, as has been found in studies of healthy school-aged children[10, 24]. With a two-sided significance of 0.05 and a power of 0.8, this required a minimum sample size of 16 for each group.

Results

Intelligence scores and sleep habits

Patients had significantly lower FSIQ than controls, and worse sleep habits, with the majority (20/22) meeting the recommended cut-off (>41) for a sleep clinic referral.

Clinical characteristics of the patient sample

These are summarised in Table 1. Twenty patients (20/22) were admitted for pre-surgical evaluation. One of these underwent invasive EEG monitoring. The remaining two patients (2/22) were admitted to guide the management of their seizures. Demographics and epilepsy characteristics of those who declined to take part were similar to those of the participants.

Diagnostic interventions and clinical events

Of those on medication, 16(72%) had this reduced or stopped. Twelve (54%) patients underwent sleep restriction at some point during the study period, half of these during the overnight study condition. Five patients (23%) had seizures during the overnight memory retention interval, and 2 (9%) during the daytime interval. Nine patients (41%) had no seizures during the study period. Four patients (18%) did not contribute data to the sleep architecture or interictal discharge rate analyses; three had their EEG electrodes removed (2 for MRI, one for early discharge) before testing could be completed, while one underwent invasive EEG monitoring and did not have adequate surface electrode data for analysis. .

Testing schedule

Due to dropouts subsequent to randomisation, of those who completed the study, nine patients were

tested under the Wake condition first, while 13 were tested under the Sleep condition first. One patient was discharged home early and returned to the hospital on a separate occasion 3 weeks later for testing under the Wake condition. The patient who underwent invasive EEG monitoring was tested 4 days (Sleep condition) and 6 days (Wake condition) after implantation respectively. Neither of these patients underwent medication reduction or sleep deprivation. For the remaining 20 patients, the average time from admission to be tested under each condition was night 2(+/-1) for the Sleep condition and day 3(+/-1) for the Wake condition. The day of testing under each condition for each patient relative to admission is detailed in Table 2.

Memory task performance

Verbal task

Memory retention was greater in the sleep condition [F(1,39)=10.8, p=0.002; Cohen's d=0.67], with no significant interaction of condition by group [F=0.03, p=0.9], and no significant main effect of group [F=2.1, p=0.1] (Fig. 2A). Co-varying for differences in baseline task performance did not alter the result (see Supplementary material for details). In separate pairwise comparisons between the sleep and wake conditions for each group, both controls (t[20]=3.0,p=0.008; Cohen's d=0.76) and patients (t[21]=2.0, p=0.06; Cohen's d=0.61) remembered more word pairs in the sleep condition. The mean gain in recall with sleep was 8.3% in controls and 7.5% in patients; on an independent samples T-test, this did not differ between the groups (p>0.8, Cohen's d=0.05). The patient with the largest decrease in memory retention with sleep (Fig. 2A) had a high overnight interictal discharge count, consistent with later findings in the correlation analysis.

Visuospatial task

Two patients were unable to co-operate on the visuospatial task due to inattention. One patient

completed testing on the verbal task over the telephone following hospital discharge, but this was not possible for the visuospatial task. Memory retention was greater in the sleep condition [F(1,36)=4.23, p=0.05, Cohen's d=0.40]. There was no significant interaction of condition by group [F=0.6, p=0.4] (Fig. 2B). In separate pairwise comparisons between the sleep and wake conditions for each group, patients remembered significantly more object locations in the sleep condition (t[18]=2.3, p=0.03; Cohen's d=0.65) but controls did not (t[20]=0.79, p=0.4; Cohen's d = 0.22). The mean gain in recall with sleep was 9.2% in patients and 4.0% in controls, and this was not different between the groups (p>0.4, Cohen's d=0.25).

The gain in memory retention with sleep was correlated for the two tasks in the patient group (R=0.53, p=0.02) but not the control group.

Thus we found that patients did not show impaired memory consolidation with sleep compared to controls. The magnitude of benefit from sleep was similar to that in controls for the verbal task and greater than that in controls for the visuospatial task.

Sleep architecture

Sleep architecture during the sleep condition memory retention interval is summarised in Table 3. Patients slept less than controls (t[30]=3.4, p=0.002), but spent similar amounts of time in light sleep (N1 and N2; p>0.6), with the deficit occurring instead in N3 (t[37]=2.1, p=0.04) and REM (t[37]=4.2,p<0.001). In the control sample, there was a strong inverse correlation between age and amount of time spent in N3 (r=-0.60, p=0.007), but this was not observed in the patient sample (r=-0.18, p=0.43). Therefore the analysis of sleep macroarchitecture suggests patients experienced more disrupted sleep during the sleep condition memory retention interval than controls.

Relationship of memory consolidation to slow wave sleep duration

Across the total sample, the gain in memory retention with sleep in the verbal task correlated with time spent in slow wave sleep (r=0.40, p=0.01)(Fig. 3). This correlation remained after controlling for age (r=0.4, p=0.03). Analysing the control and patient groups separately yielded the same correlation, but not reaching statistical significance (r=0.4, p=0.07 in controls; r=0.4, p=0.1 in patients).

Effect of epilepsy

Interictal epileptiform discharges in sleep

Four patients had no interictal discharges overnight during the sleep condition memory retention interval, six had only focal or unilateral discharges (3 left; 3 right), and eight had bilaterally independent or generalised discharges. Specific locations of IEDs for each patient are detailed in Table 2. Those with bilateral discharges tended to have higher IED loads than those with unilateral discharges only (t[9]=1.5, p=0.2, Cohen's d=0.76) (Fig. 4). The log transformed interictal discharge rate in sleep was negatively correlated to sleep-related memory consolidation in the verbal task (Rho= -0.49, p=0.037)(Fig. 4A), but this correlation did not reach significance in the visuospatial task (Rho=-0.45, p=0.08)(Fig. 4B).

Length of illness

In the verbal task, a longer duration of epilepsy was associated with poorer memory retention in the wake condition (r=-0.61, p=0.003), but not the sleep condition (r=0.13, p=0.5)(Fig. 5A). Therefore, for the verbal task, the contribution of sleep to memory consolidation increased with duration of epilepsy (r=0.53, p=0.01). In the visuospatial task, longer duration of epilepsy was associated with

poorer memory retention under the wake condition (r=-0.58,p=0.009), with a trend toward poorer memory retention in the sleep condition (r=-0.43,p=0.07)(Fig. 5B). Thus the contribution of sleep to memory consolidation in the visuospatial task also increased with duration of epilepsy, but this did not reach statistical significance (r=0.28, p=0.2, corrected p=0.8).

Subgroup analysis by seizure focus

According to the seizure semiology, imaging and video EEG telemetry findings, 11 patients had a temporal or fronto-temporal seizure focus while 11 had frontal, central, parietal, occipital or undetermined foci (see Table 1). We termed the former group "temporal" and the latter "extra-temporal".

In the verbal task, a repeated measures ANOVA with condition (wake or sleep) as the within-subjects factor and subgroup (control, temporal, extratemporal) as the between-subjects factor showed that memory retention was greater in the sleep condition (F[1, 39]= 11.2, p=0.002, Cohen's d=0.67), with no significant interaction of condition by subgroup (F=0.9, p=0.4) (Fig. 6A). In pairwise comparisons between the sleep and wake conditions for each subgroup, the temporal group remembered more word pairs in the sleep condition (t[10]=1.8, p=0.1, Cohen's d=0.86), while the extratemporal group did not (t[10]=0.8, p=0.4, Cohen's d=0.30).

In the visuospatial task, memory retention was also greater in the sleep condition (F[1,36]=3.8, p=0.05, Cohen's d=0.40), with no significant interaction of condition by group (F=0.3, p=0.7) (Fig. 6B). All of the patients (n=3) who did not complete the visuospatial task were from the extratemporal subgroup. In pairwise comparisons between the sleep and wake conditions for each subgroup, neither group showed a significant difference between the wake and sleep condition

scores ($p>0.1$).

Discussion

This study is the first to isolate the contribution of sleep to memory consolidation in children with epilepsy compared to healthy children. It is also the largest study to examine sleep and memory consolidation in patients with epilepsy to date. We showed - contrary to our primary hypothesis - that the contribution of sleep to memory consolidation in children who have focal epilepsy with a structural or presumed structural etiology is not only positive, but actually of similar magnitude to that seen in healthy children. We found the same correlation between the duration of slow wave sleep across the night and gain in memory with sleep in both groups, suggesting an intact, common underlying mechanism. Furthermore, we demonstrated this phenomenon to be robust despite a long history of illness, increasingly recruited where delayed verbal memory during waking is more impaired. Taken together, these findings suggest that sleep-related memory consolidation may serve as a compensatory mechanism to maintain cognitive function in this group of patients. Lastly, in agreement with predictions, a higher interictal discharge load during sleep was associated with lesser contribution of sleep to memory consolidation.

Sleep-related memory consolidation

Our findings appear to contradict those of smaller studies[17-19]examining sleep-related memory consolidation in children with various focal epilepsies. However, there are important differences in both the patient samples and study designs which could account for this. Urbain[18] and Galer[17] studied patients (total n=19) with primary genetic epilepsies and higher interictal discharge loads (median spike wave indices of 65 and 34 respectively) than those in our study (median spike wave index=0.7). Additionally, the lack of a delayed recall condition without sleep in these studies did not allow for the effect of sleep to be separated from the effect of delay, which is likely significant

in a sample with childhood epilepsy[25, 26]. Sud et al[19] performed their study in a similar setting, on similar patients to ours (median spike wave index=5). Seven of ten subjects remembered better in the sleep condition, however the analysed sample (n=9) was too small to show an effect of sleep. Therefore, despite differences in study design, these results, taken together with our findings, all support the idea that sleep-related memory consolidation occurs in children with epilepsy, but may be vulnerable to high interictal discharge loads in sleep. It is also possible that sleep-related memory consolidation may be impaired in the primary genetic epilepsies but not in focal epilepsies with a structural etiology, an idea supported by data from adult patients with temporal lobe epilepsy[27].

Sleep-related memory consolidation has also been examined in children with autism spectrum disorder[28] and attention deficit and hyperactivity disorder (ADHD)[29], conditions often co-morbid with epilepsy, and which may share a genetic basis[30]. Both groups of patients remembered significantly better in the sleep than the wake condition. Thus our finding that sleep-related memory consolidation is preserved in a paediatric population with neurodevelopmental disability is concordant with the wider literature.

Relationship with slow wave sleep

We found a correlation between duration of slow wave sleep (equivalent to time in N3), and the magnitude of sleep-related benefit to memory consolidation in both our control and patient samples, which was statistically significant over the total sample even when corrected for age. A correlation between sleep-related memory consolidation and N3 time has been demonstrated in adults[11] - including those with epilepsy[27], though not to our knowledge in children. Maski[28] and Prehn-Kristensen[29] examined slow wave activity (expressed as delta power in NREM); the former found no linear correlation with sleep-related memory consolidation in either healthy children or those

with autism, while the latter demonstrated a correlation in controls but not patients with ADHD. Both slow wave sleep duration and slow wave activity are markers of sleep homeostasis, but we chose to analyse the former due to its lower intra-individual variability in the face of sleep deprivation[24, 31], and lesser developmental decrease over the age range of our subjects[32]. It may be even more pertinent to quantify sleep homeostasis directly, using a measure independent of both absolute delta power and N3 time, such as that employed by Bolsterli et al[33] . They showed that sleep homeostasis is impaired in children with continuous spike waves in slow-wave sleep (CSWS), the syndrome affecting two of the four children in whom Urbain et al[18] found impaired memory consolidation over an interval of sleep. We have previously demonstrated intact within-night sleep homeostasis in a group of children with focal epilepsies with a structural etiology[34].

Relationship with nocturnal interictal discharges

We found an inverse correlation between nocturnal IED load and sleep-related memory consolidation. Galer et al[17] showed a negative correlation between spike wave index and memory performance, though only in the visuospatial domain. They also showed that the spike wave index correlated highly with the diffuseness of interictal discharges. Similarly, those cases in our sample with the greatest IED loads tended to have bilateral discharges. It is notable that all but two of our patients showed sleep benefit within the range seen in healthy controls, even though just four were free of IEDs. This suggests it may be possible to elucidate a threshold below which interictal discharges in sleep have no significant impact on cognition and hence would not warrant treatment. There is evidence this threshold may lie between spike wave indices of 1 and 10[35, 36] regardless of underlying etiology, and our current findings are consistent with this.

Relationship with duration of epilepsy

Early age of onset[37] and long duration[38] of epilepsy are known to be associated with a high risk of cognitive impairment in general, particularly where there is drug resistance[37], as in our sample. While the wake condition memory retention scores showed a strong correlation with duration of epilepsy, the sleep condition scores did not, suggesting some protective or compensatory effect of sleep on memory retention. This was more marked (reaching statistical significance) in the verbal task, consistent with this task benefiting strongly from sleep in both patients and controls.

Relationship with seizure focus

When assessed with standard psychometric tests, children with temporal lobe epilepsy show greater memory impairment - particularly in the verbal domain - than children with frontal seizure foci[38]. In our sample, memory retention in the Wake condition - which is similar to standard tests of delayed verbal recall - was poorer in the 'temporal' subgroup, yet the benefit of sleep to memory consolidation was greater, thus supporting the idea of a robust compensatory mechanism.

Limitations

The main weakness of our study was the lack of patients with moderate to high interictal discharge loads in sleep, limiting our examination of the relationship between sleep-related memory consolidation and interictal epileptiform discharges. However this is typical for a group of children with focal epilepsies with a structural etiology, and we were still able to demonstrate a linear relationship. The heterogeneity of our patient sample meant that once broken into subgroups by underlying pathology, medication or seizure burden, numbers became too small for meaningful analysis. Conversely, this clinical diversity increases the generalizability of our results to children with such focal epilepsies. The hospital setting and clinical interventions may have disrupted patients' sleep, though one would have expected an adverse effect on sleep-related memory

consolidation. There is in fact evidence that neither setting[17] nor acute sleep disruption[24] significantly influence performance on delayed memory tasks. Patients undergoing long-term video EEG telemetry for pre-surgical evaluation tend to accumulate clinical interventions and ictal events over the course of their admission. To minimise the influence of this on memory task performance, we counterbalanced the order of the sleep and wake conditions across participants. The first testing was performed as soon as possible after consent was obtained, and the second testing as soon as possible with at least a 24-hour interval from the first.

Conclusion

Using a controlled, within-subject design with adequate power to detect an effect of sleep on memory, we have demonstrated for the first time that sleep-related memory consolidation is intact in a group of children with epilepsy.

By isolating the contribution of sleep to memory in a sample with ongoing seizures, poor sleep habits and acute sleep disruption, our results suggest that sleep-related memory consolidation is an extremely robust mechanism. This is an important positive finding for clinicians and patients worldwide, who can readily harness it to aid learning - for instance, by recapping each day's new facts before bed - even where sleep appears severely disturbed.

In terms of advancing the argument that sleep disruption may contribute to cognitive impairment, our findings support nocturnal interictal discharges as the most likely causative factor, and suggest that what is disrupted cannot be measured at the level of sleep macroarchitecture. Further studies should include patients with a full range of nocturnal interictal discharge loads, examining the

impact on sleep microarchitecture[39] and homeostasis, and relating this to concurrent cognitive measures.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure legends

Figure 1. Study design. Each participant performed a verbal and a visuospatial task under two conditions, Wake (top) and Sleep (bottom) , The order of conditions was balanced across participants. For each condition, material was learned to a criterion level, and following an 8 to 15 hour interval, recall was tested. EEG/polysomnography was performed across the Sleep condition.

Figure 2. Memory task performance. Memory retention scores, calculated as the percentage difference in items recalled at testing compared to criterion. In the verbal task (A), Memory retention was greater in the sleep condition ($p=0.002$), with no significant interaction of condition by group. In the visuospatial task (B), memory retention was greater in the sleep condition ($p=0.05$), with no significant interaction of condition by group.

Figure 3. Sleep benefit and duration of N3. Across the total sample, sleep benefit - calculated as the difference between the sleep and wake condition memory retention scores - for the verbal task correlated with time spent in N3 ($r=0.4$, $p=0.01$). This correlation remained after controlling for age ($p=0.03$), and was the same in each group (patients: $r=0.4$, $p=0.1$; controls $r=0.4$, $p=0.07$)

Figure 4. Effect of interictal discharges on sleep benefit. Sleep benefit was calculated as the difference between the sleep and wake condition memory retention scores. Patients with focal or unilateral discharges only are indicated in orange while patients with bilateral or generalised discharges are indicated in blue. In the verbal task (A), a higher rate of interictal discharges was associated with lesser gain in memory retention with sleep ($p=0.04$). In the visuospatial task (B) this correlation did not reach significance ($p=0.08$). SWI = spike wake index; transformation used = $\log10(1+x)$.

Figure 5. Effect of duration of epilepsy on memory retention. In the verbal task (A), a longer duration of illness was associated with poorer memory retention in the wake condition (p=0.003), but no change in memory retention in the sleep condition (p=0.5). In the visuospatial task (B), a longer duration of illness was associated with poorer memory retention in both conditions (wake condition: p=0.009, sleep condition: p=0.07).

Figure 6. Memory task performance by temporal/ extra-temporal seizure focus. Memory retention scores, calculated as the percentage difference in items recalled at testing compared to criterion. In the verbal task (A), Memory retention was greater in the sleep condition (p=0.002), with no significant interaction of condition by group. Post-hoc pairwise comparisons of performance in the Sleep and Wake conditions show a large effect of sleep on memory consolidation in the 'temporal' subgroup (t[10]=1.8, p=0.1, Cohen's d=0.86) but not the 'extra-temporal' subgroup (t[10]=0.8, p=0.4, Cohen's d=0.30) . In the visuospatial task (B), memory retention was greater in the sleep condition (p=0.05), with no significant interaction of condition by group.

	Patients (n=22)	**Controls (n=21)**	**p**
Demographics, sleep habits, handedness			
Age (years; mean +/-SD)	11.5 (+/-3.0)	10.6(+/-2.8)	0.34
Sex (females, males)	8,14	12,9	0.18
FSIQ (mean +/-SD)	88.4(+/-11.3)	115.3(+/-12.9)	<0.001
CSHQ (mean +/-SD)	49.6 (+/-9.6)	37.4(+/-3.8)	<0.001
Right handed	19 (86%)	19 (90%)	1
Epilepsy characteristics			
Age of onset (years)(mean +/-SD)	5.3(+/-4.1)		
Duration of epilepsy (years)(mean +/-SD)	6.1(+/-2.8)		
MRI findings			
No lesion	7 (32%)		
Focal cortical dysplasia	7 (32%)		
Mesial temporal sclerosis	2 (9%)		
Low grade tumour	2 (9%)		

Other lesion	4 (18%)
Seizure frequency	
Daily	5 (22%)
Weekly	9 (41%)
Monthly	3 (14%)
<1 per month	5 (23%)
Seizure lateralisation	
Left	6 (27%)
Right	5 (23%)
Bilateral	3 (14%)
Undetermined	8 (36%)
Seizure localisation	
Frontal	3 (13%)
Temporal	6 (27%)
Fronto-temporal	5 (23%)
Central	1 (5%)
Parietal	1 (5%)
Undetermined	6 (27%)
Number of anti-epileptic drugs	
0	2 (9%)
1	10 (45%)
2	7 (32%)
3	3 (14%)
Type of anti-epileptic drug	
Levetiracetam	8 (36%)
Sodium valproate	7 (32%)
Carbamazepine	6 (27%)
Lamotrigine	4 (18%)
Clobazam	3 (14%)
Oxcarbazepine	2 (9%)
Topiramate	2 (9%)
Lacosamide	1 (5%)

Table 1. Participant characteristics. SD = standard deviation; FSIQ = full scale intelligence quotient; CSHQ = Children's Sleep Habits Questionnaire score. Seizures were lateralised and localised by semiology, imaging and video EEG. Figures for anti-epileptic drugs used do not add up to 100% due to polypharmacy.

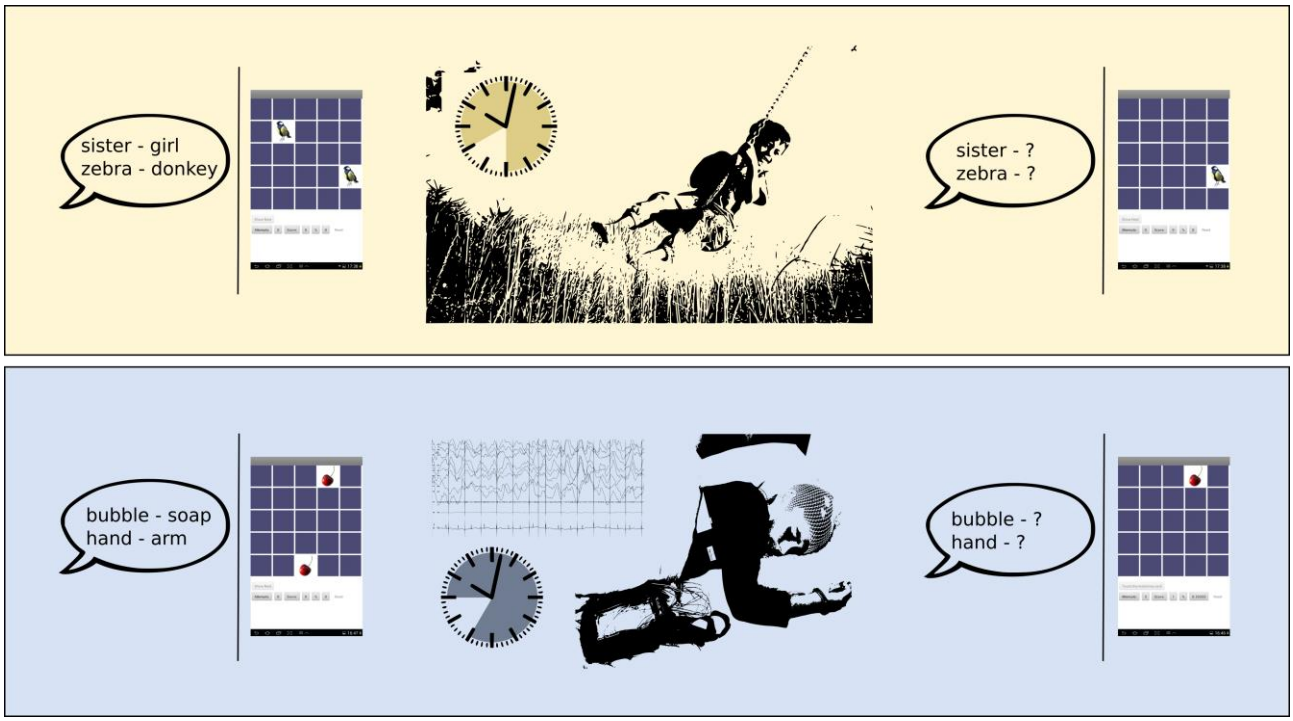
	Patients (n=18)	Controls (n=21)	p
TST	477 (+/-104)	566 (+/-51)	0.002
WASO	24 (+/- 19)	28 (+/-34)	0.7
N1	34 (+/- 17)	36 (+/-19)	0.6
N2	192 (+/-80)	178 (+/-47)	0.6
N3	162 (+/-56)	205 (+/-60)	0.04
REM	88(+/-45)	147 (+/-34)	<0.001

Table 3. Sleep architecture during overnight memory retention interval. All quantities are in minutes and presented as mean (+/- standard deviation). TST = total sleep time; WASO = wake after sleep onset; N1, N2, N3 = deepening stages of NREM sleep; N3 is equivalent to slow wave sleep. REM= rapid eye movement sleep. WASO includes nocturnal seizures (n=5 Patients). Independent sample t-tests (Welch) were used to compare parameters between patients and controls.

Patient No.	IED location	Sleep	Wake
1*	F7, F3	4	3
2	O1	3	2
3	F4	2	4
4	P8	2	4
5	C6, P8	4	3
6*	F4, F3	1	3
7	F4, F8	3	2
8	F7, F9, T7	1	3
9	F4, F8	1	2
10	C5, C6	2	23
11	F9, F7, F9	3	2
12	P8, P4	1	3
13	F4, C4	2	4
14	T7. T9	1	3
15	F8, F10	2	4
16*	RAD1, LHD1	5	7
17	F4	1	3
18	T7, F9	3	2
19	F4	1	3
20	Fz, F3	4	2
21*	C3	4	2
22	F3,C3	4	2

Table 2. Locations of the predominant focal interictal discharges for each patient, and time of testing (number of days after admission) for the Sleep and Wake conditions. Patients who did not contribute data to the interictal discharge rate analysis are marked with an asterisk '*'. Patient 10 was discharged home on day 3, but returned for testing in the Wake condition three weeks later.

Patient 16 underwent invasive EEG monitoring and was implanted on day 1 of admission; RAD = right amygdala depth electrode, LHD = left hippocampal depth electrode.


sister - girl
zebra - donkey
sister - ?
zebra - ?
bubble - soap
hand - arm
bubble - ?
hand - ?
Figure 1

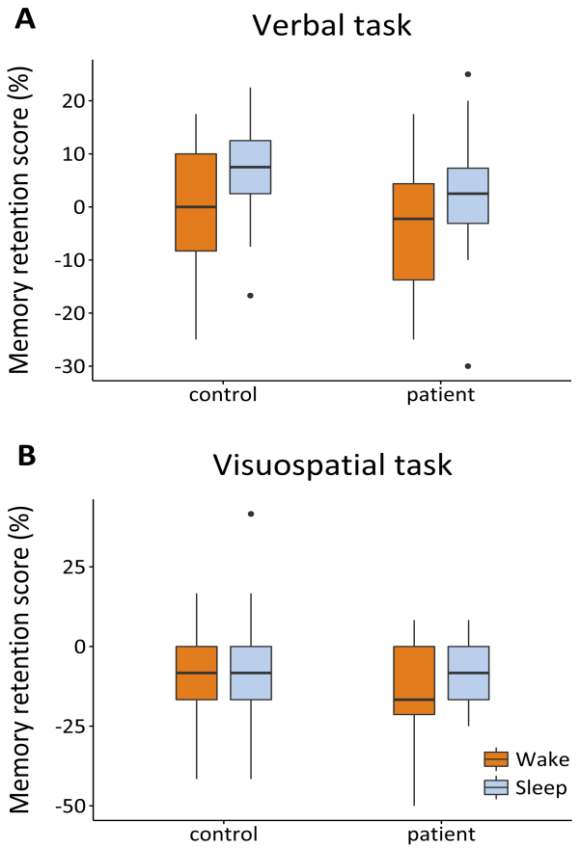

A
Verbal task
Memory retention score (%)
20
10
0
-10
-20
-30
control
patient
B
Visuospatial task
Memory retention score (%)
25
0
-25
-50
control
patient
Wake
Sleep

Figure 2

Figure 3

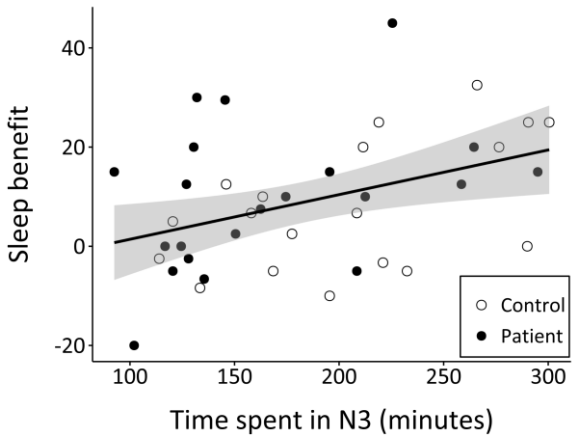

Sleep benefit
40
20
0
-20
100
150
200
250
300
Time spent in N3 (minutes)
Control
Patient

Figure 4

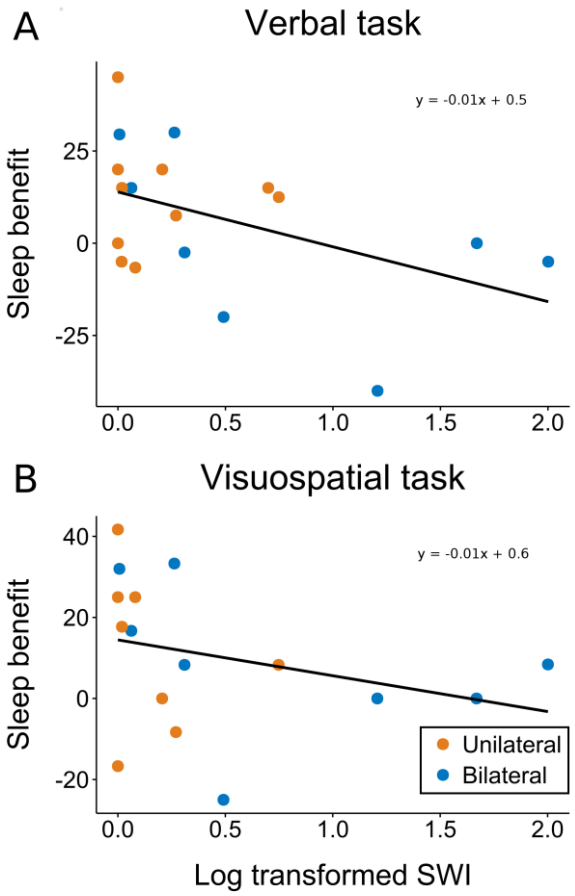

A
Verbal task
y = -0.01x + 0.5
Sleep benefit
25
0
-25
0.0
0.5
1.0
1.5
2.0
B
Visuospatial task
y = -0.01x + 0.6
Sleep benefit
40
20
0
-20
Unilateral
Bilateral
0.0
0.5
1.0
1.5
2.0
Log transformed SWI

Figure 5

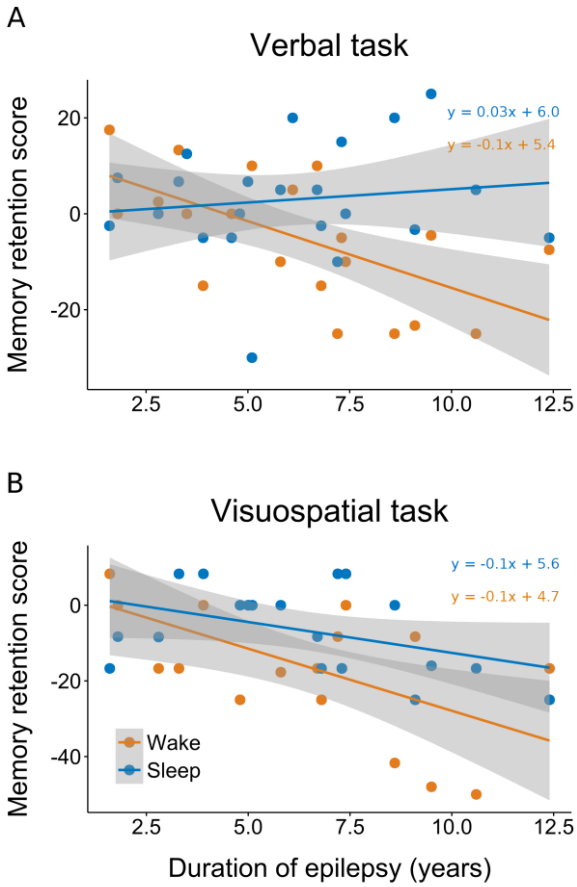
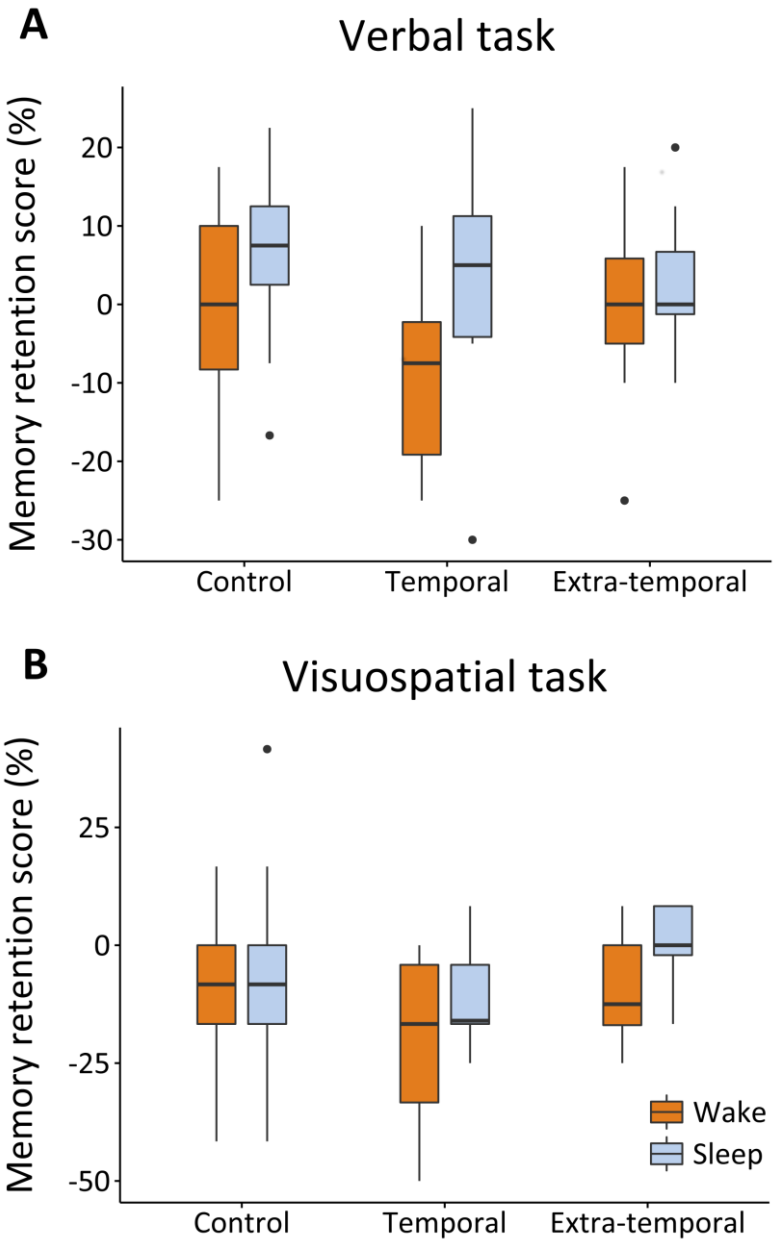

A
Verbal task
Memory retention score
y = 0.03x + 6.0
y = -0.1x + 5.4
20
0
-20
2.5
5.0
7.5
10.0
12.5
B
Visuospatial task
Memory retention score
y = -0.1x + 5.6
y = -0.1x + 4.7
0
-20
-40
Wake
Sleep
2.5
5.0
7.5
10.0
12.5
Duration of epilepsy (years)

Figure 6


A
Verbal task
Memory retention score (%)
20
10
0
-10
-20
-30
Control
Temporal
Extra-temporal
B
Visuospatial task
Memory retention score (%)
25
0
-25
-50
Control
Temporal
Extra-temporal
Wake
Sleep