

Keeping time in your sleep: Overnight consolidation of temporal rhythm

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ABSTRACT

Temporal processing forms the basis of a vast number of human behaviours, from simple perception and action to tasks like locomotion, playing a musical instrument, and understanding language. Growing evidence suggests that these procedural skills are consolidated during sleep, however investigation of such learning has focused upon the order in which movements are made rather than their temporal dynamics. Here, we use psychophysics and neuroimaging to explore the possibility that temporal aspects of such skills are also enhanced over a period of sleep. Behaviourally, our examinations of motor (tapping a finger in time with a temporal rhythm) and perceptual (monitoring a temporal rhythm for deviants) tasks reveal post-sleep improvements in both domains. Functionally, we show that brain-state during retention (sleep or wake) modulates subsequent responses in the striatum, supplementary motor area, and lateral cerebellum during motor timing, and in the posterior hippocampus during perceptual timing. Our data support the proposal that these two forms of timing draw on different brain mechanisms, with motor timing using a more automatic system while perceptual timing of the same rhythm is more closely associated with cognitive processing.

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1. Introduction

Humans spend a large proportion of their waking lives performing skilled tasks which require little attention. These include movement based activities like walking or riding a bicycle and perceptual activities like reading, understanding speech, or judging the depth of a visual scene. Careful analysis has demonstrated that many of these tasks can be broken down into independently learned temporal and ordinal elements (Ullen & Bengtsson, 2003). Furthermore, many such procedural skills have been shown to improve across periods of sleep (Born, Rasch, & Gais, 2006; Dang-Vu, Desseilles, Peigneux, & Maquet, 2006; Fenn, Nusbaum, & Margoliash, 2003; Fischer, Drosopoulos, Tsen, & Born, 2006; Fischer, Hallschmid, Elsner, & Born, 2002; Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Laureys, Peigneux, Perrin, & Maquet, 2002; Maquet et al., 2000; Maquet, Schwartz, Passingham, & Frith, 2003; Plihal & Born, 1997; Rauchs, Desgranges, Foret, & Eustache, 2005; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000; Walker & Stickgold, 2004; Walker & Stickgold, 2006). Most examinations of such off-line enhancement have focussed on the order

of responses (Cohen, Pascual-Leone, Press, & Robertson, 2005; Robertson, Pascual-Leone, & Press, 2004; Spencer, Sunm, & Ivry, 2006; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002; Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005). Consolidation of the temporal aspects of these tasks therefore remains largely unexplored, though one study (Maquet et al., 2003) demonstrated that the emergent timing in visuomotor tracking is strengthened over sleep. In the current report, we aim to build on this work by determining whether the event timing in skilled rhythm processing (Spencer, Zelaznik, Diedrichsen, & Ivry, 2003; Zelaznik, Spencer, & Doffin, 2000; Zelaznik, Spencer, & Ivry, 2002) also undergoes overnight consolidation, and to examine the neuroplasticity associated with such changes.

Studies of procedural learning have shown that the brain areas in which responses are enhanced as learning progresses (Doyon et al., 2009) commonly exhibit greater activity when the task is performed after sleep. This is true for both motor (Albouy et al., 2008; Walker, Stickgold, Alsop et al., 2005) and perceptual (Walker, Stickgold, Jolesz, & Yoo, 2005) forms of learning. A wide range of evidence suggests that different temporal processing mechanisms are recruited in different forms of timing task (Lewis & Miall, 2003; Rammsayer, 1999; Wiener, Turkeltaub, & Coslett, 2010; Zelaznik et al., 2002). Meta-analysis of neuroimaging studies of time perception (Lewis & Miall, 2003) shows that areas of the motor system, including striatum, cerebellum, and SMA are recruited for tasks in which timing is performed more or less automatically (e.g.,

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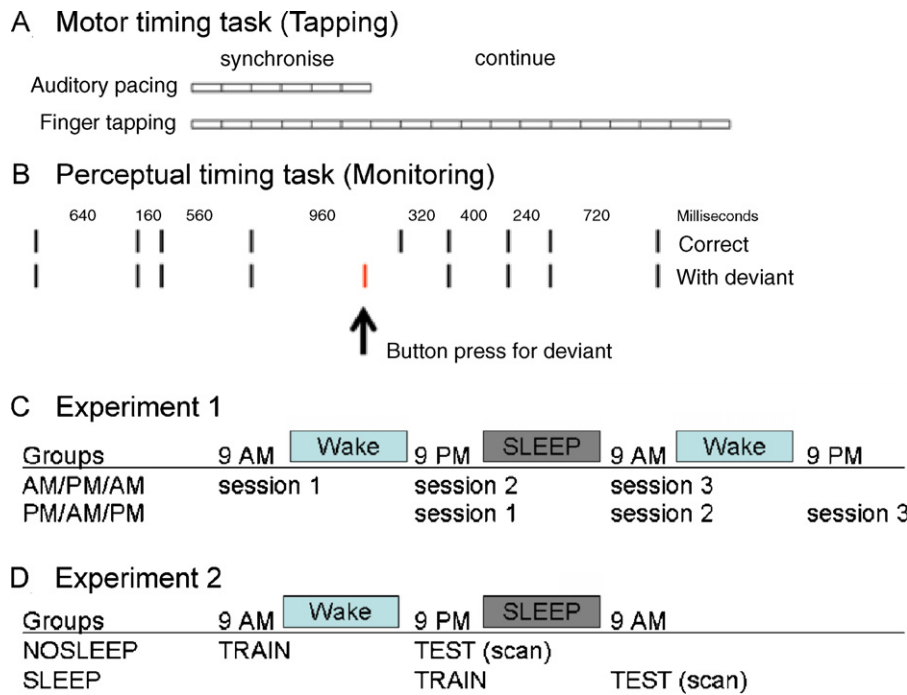


Fig. 1. Tasks and paradigms. (A) The tapping task: in each trial, participants tapped their right index finger in time with an auditory rhythm, then continued to tap the same temporal rhythm once the auditory cues had ceased. Each oblong box represents a repetition of the full rhythm sequence (or 'bar'). (B) The Monitoring task: participants listened to a repeating auditory rhythm and pressed a key when they detected a temporal deviant. Deviants were rare and consisted of an auditory beep which occurred 200 ms too early or too late, disrupting the timing of the two adjacent intervals in the rhythm. (C) The testing schedule for AM/PM/AM and PM/AM/PM groups in Experiment 1. (D) The testing schedule for sleep and wake groups in Experiment 2.

continuous, repetitive motor timing of intervals less than one second), while prefrontal and parietal regions are recruited for the more cognitively demanding timing in tasks with the opposite characteristics (e.g., discontinuous, non-motor timing of intervals greater than one second). Because many tasks encompass a combination of these characteristics, it has been proposed that elements of both timing systems can be recruited simultaneously (Lewis & Miall, 2003). The distinct yet potentially overlapping brain regions recruited during motor and perceptual timing provide a prime example of this (Buetti, Walsh, Frith, & Rees, 2008; Clarke, Ivry, Grinband, & Shimizu, 1996; Lewis & Miall, 2003; Wiener et al., 2010). Here, we explore the distinction between motor and perceptual timing by studying offline consolidation of brain activity during both types of task.

To allow examination of both motor and perceptual timing, we elected to use auditory rhythms akin to those found in music as our stimuli. In our motor paradigm, 'Tapping' (Wing & Kristofferson, 1973), participants synchronised button presses with a rhythm of auditory beeps which occurred in a repeating temporal pattern, then continued to tap the same temporal rhythm without external cues (Fig. 1A). In our perceptual paradigm, 'Monitoring', participants listened to an equivalent auditory rhythm and monitored it for rare temporal deviants (Fig. 1B), pressing a button when these were detected. We performed two experiments using these paradigms: one which examined the interaction between sleep and behavioural performance in the Tapping task, and a second which used functional magnetic resonance imaging (fMRI) to monitor sleep-related alterations in the brain responses associated with both Tapping and Monitoring tasks.

Based upon a prior analysis of the systems used in automatic and cognitively controlled timing (Lewis & Miall, 2003), we expected consolidation across sleep to modulate learning-related alterations in brain response within the movement control system (particularly the supplementary motor area (SMA), cerebellum, and striatum) during motoric rhythm tapping (Wiener et al., 2010), and

in higher cognitive areas (potentially including prefrontal and parietal cortices) during perceptual rhythm Monitoring (Lewis & Miall, 2006).

2. Materials and methods

2.1. Participants

All participants were consenting, healthy, right handed, and had no history of psychiatric illness. 14 of these (7 male and 7 female, mean age 27) participated in Experiment 1, and 24 (12 male and 12 female, mean age 25, \pm SEM 1 year) in Experiment 2. All participants were instructed to abstain from alcohol, caffeine, and other drugs during, and for twenty-four hours prior to, the experiment. Experiments were approved by the Liverpool research ethics committee.

2.2. Behavioural tasks

2.2.1. Experiment 1: behavioural testing

Participants performed a motor synchronisation continuation task (Wing & Kristofferson, 1973) (Fig. 1A, Tapping) in three sessions. The rhythm they learned was based on those in Lewis, Wing, Pope, Praamstra, and Miall (2004) and consisted of eight temporal intervals: 107, 429, 214, 1065, 536, 643, 321, and 857 ms, in that order, with each bar (repeating sequence of intervals) lasting 4172 ms. Each trial was initiated by a press to the spacebar, and contained a synchronisation phase immediately followed by a continuation phase. During synchronisation, the rhythm was presented via auditory beeps (250 Hz for 25 ms) and was repeated 6 times (25 s total), during continuation auditory presentation stopped and participants were exposed to 47 s of silence, terminated by a high pitched beep. Participants were instructed to synchronise right index finger button presses with the beeps during synchronisation (6 bars), then continue to press the button in the same temporal sequence during continuation (11 bars). There were 8 synchronisation/continuation trials in every session.

Fourteen participants were randomly divided into two groups, determined by the time of day at which they were trained and tested: an AM/PM/AM group (seven participants) and a PM/AM/PM group (seven participants). In the AM/PM/AM group, Session 1 was performed in the morning of day 1, Session 2 that evening, and Session 3 in the morning of day 2 (Fig. 1C). In the PM/AM/PM group, the order was reversed: Session 1 was performed on the evening of Day 1, Session 2 the next morning (Day 2), and Session 3 that evening. For these two groups, all experimental sessions began between 8:00 and 11:00 AM or 20:00 and 23:00 PM, and delays between sessions were always 12 h (\pm 1).

2.2.2. Experiment 2: functional imaging

Participants in Experiment 2 were divided into sleep and wake groups, each of these comprising 6 males and 6 females (12 participants total per group, 24 total in the experiment). Sleep participants were trained in the evening and scanned next morning, wake participants were trained in the morning and scanned that evening (Fig. 1D). Each group was instructed to go about their normal routine (e.g., going to work, class, or sleep as usual). The wake group was instructed to abstain from daytime napping.

The Tapping task in Experiment 2 was similar to that used in Experiment 1, but implemented two different rhythm sequences (A and B). Intervals in these rhythms were: 640, 160, 560, 960, 320, 400, 240, and 720 ms in A, and 320, 1040, 800, 160, 240, 400, 480, and 560 ms in B, and were presented in those orders using auditory beeps (again 250 Hz and 25 ms duration) with a repeating bar of 4000 ms. Participants attended a Training session and a Scanning session that were separated by 12 h (± 1). All experimental sessions began between 8:00 and 11:00 AM or 20:00 and 23:00 PM.

The first session, Training, was performed outside the MRI scanner. Participants learned either rhythm A or B (Learned rhythm, counterbalanced across participants) via a series of trials in which they listened to an auditory presentation of the rhythm and synchronised right index finger button presses to it for 6 bars (synchronisation, 24 s), then continued to press the button in the same temporal rhythm in silence for a further 12 bars (continuation, 48 s). Continuation was terminated by a high-pitched beep. Each training session contained 8 trials of synchronisation followed by continuation, and each trial was initiated by a press to the spacebar. The second session, Scanning, was performed inside the MRI scanner. Learned and Unlearned rhythms were interleaved in 8 alternating trials, with each trial comprising 8 bars (repetitions) of synchronisation and 5 bars of continuation. These rhythm trials were randomly interleaved with 20 s epochs of a fixation baseline. Visual cues specifying 'rest', 'synchronise', or 'continue', were presented as appropriate.

In addition to the motor synchronisation/continuation task (Tapping, Fig. 1A), participants performed a perceptual monitoring task (Monitoring, Fig. 1B) during the scanning session of Experiment 2. In Monitoring, participants listened to rhythms A and B (Learned and Unlearned) in 10 alternating trials of 32 s, and responded by pressing a button with the right index finger when a beep was misplaced in time by 200 ms (deviation). A maximum of 3 deviations occurred in any trial, and these could not occur within the first two bars of the rhythm. Participants practiced this task for two trials before the start of scanning to become familiar with the Unlearned rhythm. As with Tapping, trials of Monitoring were randomly interleaved with 20 s epochs of fixation baseline and visual cues specifying 'rest' or 'monitor' were presented as appropriate.

The same Learned and Unlearned rhythms were used for Monitoring as Tapping, but Monitoring was performed only at Scanning (not in training). This meant that monitored rhythms were learned via the synchronisation/continuation Tapping paradigm in the Training session. Participants performed both Monitoring and Tapping tasks in the fMRI scanner and the order in which these were performed was counterbalanced across individuals, with data for each task collected in a separate scanning session (run).

2.3. Behavioural analysis

Performance on Tapping was assessed during the continuation phase. Each sequence of button presses was aligned with the presented rhythm using the longest and shortest intervals according to the method in Lewis et al. (2004), and those estimates differing from the target interval by >95% were excluded as outliers. For each trial, the coefficient of variation (CV) for each target interval was calculated by dividing the mean of estimates by the standard deviation of estimates. CV's from all 8 target intervals were then averaged to give a single measure for the trial. In the Monitoring task, behavioural performance was measured as Performance = (Hits - False Alarms) / total number of actual deviants, where false alarms included button presses <1 s after the deviant. Normality of behavioural data was confirmed using a Kolmogorov-Smirnov test prior to further statistical analysis.

2.4. Equipment and fMRI parameters

The behavioural paradigms for both experiments were written in Cogent on a Matlab 6.5 base. We used a Domino 2 system from Micromint to log responses with accuracy ~1 ms. During fMRI scanning, we used an MR compatible audio setup from MR Confon to present auditory stimuli.

Functional imaging was performed on a 3-T Trio MR scanner (Siemens Vision, Erlangen, Germany) with an 8 channel head coil. We used echo-planar imaging to obtain image volumes with 31 contiguous oblique transverse slices every 2 s (voxel size 3.5 mm \times 3.5 mm \times 2 mm, 80% gap, TE 30 ms) covering the whole brain. Data for Tapping and Monitoring were collected in separate sessions (runs).

2.5. Functional analysis

Functional MRI images were analysed using the statistical parametric mapping (SPM2) software package (Wellcome Department of Cognitive Neurology, London, UK, <http://www.fil.ion.ucl.ac.uk/spm/>). The functional volumes from each session were corrected for head motion by realigning with the first image, spatially nor-

malised to an EPI template corresponding to the Montreal Neurological Institute (MNI) space, and smoothed using a Gaussian Kernel size of 5 mm full-width at half-maximum.

To characterise functional responses, the data were examined using a 2-level random-effects analysis. First level (within participant) analyses were performed separately for Tapping and Monitoring since these data were acquired in separate sessions. In this analysis, the responses of individual participants were examined using an individual first-level design matrix. For Tapping, this model included four main regressors: Learned synchronisation, Learned continuation, Unlearned synchronisation, and Unlearned continuation. To reduce noise associated with individual variation in motor performance, CV (calculated using the 20 s of continuation data in each trial), was included as a parametric regressor of no interest for each continuation block. For Monitoring, the first-level design matrix included four regressors: Learned and Unlearned rhythm trials, deviant presentation times, and button-press response times. To control for motion artefacts, six ridged body movement parameters were included as regressors of no interest in each design matrix. Parameter estimates reflecting the height of the hemodynamic response function for each regressor were calculated at each voxel. Contrast images providing a direct comparison of responses in Learned and Unlearned conditions were then calculated for both Tapping and Monitoring. The resulting images were used in a second-level random effects analyses that combined data across participants.

In order to isolate learning-related changes in activation which developed across sleep, the interaction Sleep [Learned > Unlearned] <> Wake [Learned > Unlearned] was calculated. Comparison of Learned and Unlearned rhythms at the subject level served two functions. First, it controlled for circadian factors by removing activities associated with retrieval at a specific time of day, following the method of Walker, Stickgold, Alsop, et al. (2005). Second, it allowed isolation of learning-related responses. The contrast images resulting from this subtraction were used to form a second-level one-way ANOVA which compared sleep and wake conditions. This analysis was performed separately for Tapping (*contrast 1*) and Monitoring (*contrast 2*).

To determine how overnight changes differed between the two tasks, the interaction terms generated by contrasts 1 and 2 were compared directly. This was achieved by forming a second level ANOVA with the two level factors: Tapping (sleep and wake), and Monitoring (sleep and wake). The interaction between task and group (*contrast 3*) was used to identify regions where modulation of learning-related responses by brain state (sleep/wake) during retention differed significantly between tasks. This was calculated as: Tapping [Sleep (Learned > Unlearned) > Wake (Learned > Unlearned)] <> Monitoring [Sleep (Learned > Unlearned) > Wake (Learned > Unlearned)]. Finally, an SPM conjunction (Friston, Penny, & Glaser, 2005) was used to test for regions brain state during retention modulated responses in both tasks (*contrast 4*).

Responses in contrasts 1–4 were considered significant at $p < 0.001$ uncorrected and a cluster size of $k = 5$ voxels. To test for correlations between consolidation-related improvements in performance and neural activity, we extracted the parameter estimates associated with the contrast Learned–Unlearned for the group peak voxels in hippocampus, striatum, SMA, and dorsal cerebellum for each participant, and regressed these against improvement in performance (Train CV–Test CV) for Sleep and wake groups.

3. Results

3.1. Experiment 1: behavioural testing

In Experiment 1, performance was assessed using a 2 \times 2 mixed analysis of variance (ANOVA) with delay type (sleep/wake) as the within subject factor, group (AM/PM/AM or PM/AM/PM as determined by the time of day of the testing sessions) as the between subject factor, and the difference between CVs across offline delay periods (Delay 1: [Session 1 – Session 2], and Delay 2: [Session 2 – Session 3]) as the dependent variable. This identified a main effect of delay type (ANOVA $F(1,12) = 9.94$, $p < 0.008$), but no effect of group, and no interaction. Post-hoc two-tailed t -tests between adjacent sessions revealed improvement over delays containing sleep, but not over equivalent delays containing only wakefulness. Specifically, in the AM/PM/AM group, there was an overnight improvement ($M = 27\% \pm 12\%$ SEM) from the PM to the subsequent AM session ($t(12) = 2.44$, $p = 0.031$), and in the PM/AM/PM group there was an overnight improvement ($M = 32\% \pm 7\%$ SEM) from the AM to the subsequent PM session ($t(12) = 2.57$, $p = 0.029$), see Fig. 2A and B. Since performance improved only after periods of sleep, and considering that these improvements were maintained in subsequent sessions, these results indicate that learned representations of temporal rhythm were modulated offline, across a night of sleep.

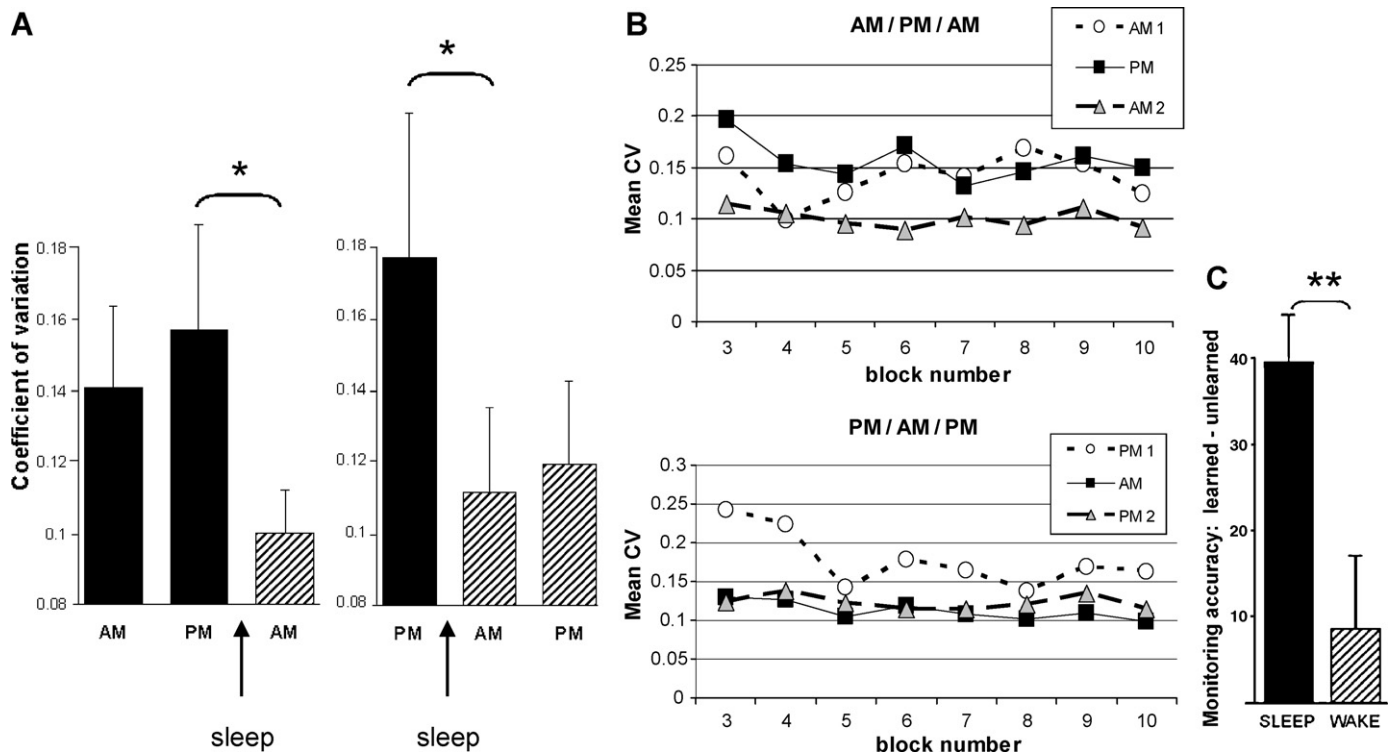


Fig. 2. Behavioural performance. (A) Behavioural results from Experiment 1 pooled across all trials in a session. From L to R results are shown for the AM/PM/AM and PM/AM/PM. Comparison of performance over the two 12 h consolidation periods showed a significant decrease in CoV after each epoch of sleep, but not after equivalent epochs of wakefulness (t -test $p = 0.03$ for both groups). (B) To further illustrate these results, data from the AM/PM/AM and PM/AM/PM groups are shown in a trial-by-trial basis (each point represents a group mean). (C) Behavioural results from the Monitoring task in Experiment 2. The figure demonstrates a marked improvement in Monitoring performance after sleep but not after a similar period of wakefulness. A 2-tailed t -test confirms that the difference in Monitoring percent correct [Learned–Unlearned] is greater in SLEEP than WAKE ($p = 0.006$). Error bars show one SEM.

To determine whether there was an interaction between time of day and tapping performance, a paired two-tailed t -test was used to compare mean CV for session 1 across the two groups (AM/PM/AM and PM/AM/PM). This revealed no significant difference in performance on initial training in the AM and PM sessions ($t(12) = 0.53$, $p = 0.481$). As a second control for circadian influences upon performance, CVs in the first and last session (24 h later) were compared using a 2×2 ANOVA with the factors group and session (first/last). This revealed a main effect of session, ($F(1,12) = 7.63$, $p = 0.017$), demonstrating that performance by the same participant was improved at retest 24 h after the first session irrespective of whether this occurred in the morning or evening (Fig. 2A). There was no interaction between group and session (first/last) ($F(1,12) = 0.21$, $p = 0.656$), showing that the time of day at which participants were trained or tested did not impact upon performance 24 h later. The normality of data from Experiment 1 was confirmed using a Kolmogorov–Smirnov test.

Overall, the behavioural data from Experiment 1 show marked improvement in tapping performance after a retention interval containing sleep, but not after an equivalent interval containing only wakefulness. Furthermore, these enhancements in performance were shown to be independent of diurnal test time.

3.2. Experiment 2: functional imaging

3.2.1. Behavioural results

In Experiment 2, offline changes in Monitoring behaviour were assessed using a 2×2 mixed ANOVA with the factors sequence (Learned/Unlearned), and group (sleep/wake), and with performance (see Section 2) as the dependent variable. This revealed a performance benefit for Learned sequences (main effect of learning, $F(1,22) = 22.76$, $p < 0.001$), with a specific facilitation of this benefit

in those who had slept (interaction between sequence and group, ($F(1,22) = 9.37$, $p = 0.006$, see Fig. 2C). This overnight improvement suggests that the consolidation of timing behaviour observed in Experiment 1 is not merely associated with the motor components of tapping a rhythm, but also generalises to the perceptual domain.

Offline changes in Tapping performance on the learned sequences in Experiment 2 were assessed using a 2×2 mixed ANOVA with the factors session (session 1/session 2) and group (sleep/wake), and with CV as the dependent variable. This showed no significant results ($F(1,22) = 0.93$; $p = 0.346$). As an alternate way of examining the impact of consolidation, a second 2×2 mixed ANOVA tested for differences in performance of Learned and Unlearned sequences during fMRI scanning. The factors were sequence (Learned/Unlearned) and group (sleep/wake). This also failed to reveal significant results (main effect of sleep $F(1,22) = 0.78$; $p = 0.387$). As with Experiment 1, the normality of behavioural data in Experiment 2 was confirmed using a Kolmogorov–Smirnov test.

Overall, the behavioural results from Experiment 2 provide evidence that a centralised (nonmotor) representation of the learned rhythm is strengthened across a night of sleep. Interestingly, this strengthened representation was indexed by enhanced performance on Monitoring, but not Tapping. The possibility that this difference between tasks may be due to difficulties that the noisy environment and constrained physical position associated with the fMRI scanner posed for skilled performance of the Tapping task is considered in the discussion.

3.2.2. fMRI results

In Tapping, the interaction analysis [Sleep(Learned > Unlearned) > Wake(Learned > Unlearned)] (contrast 1) tested for regions where learning related changes in

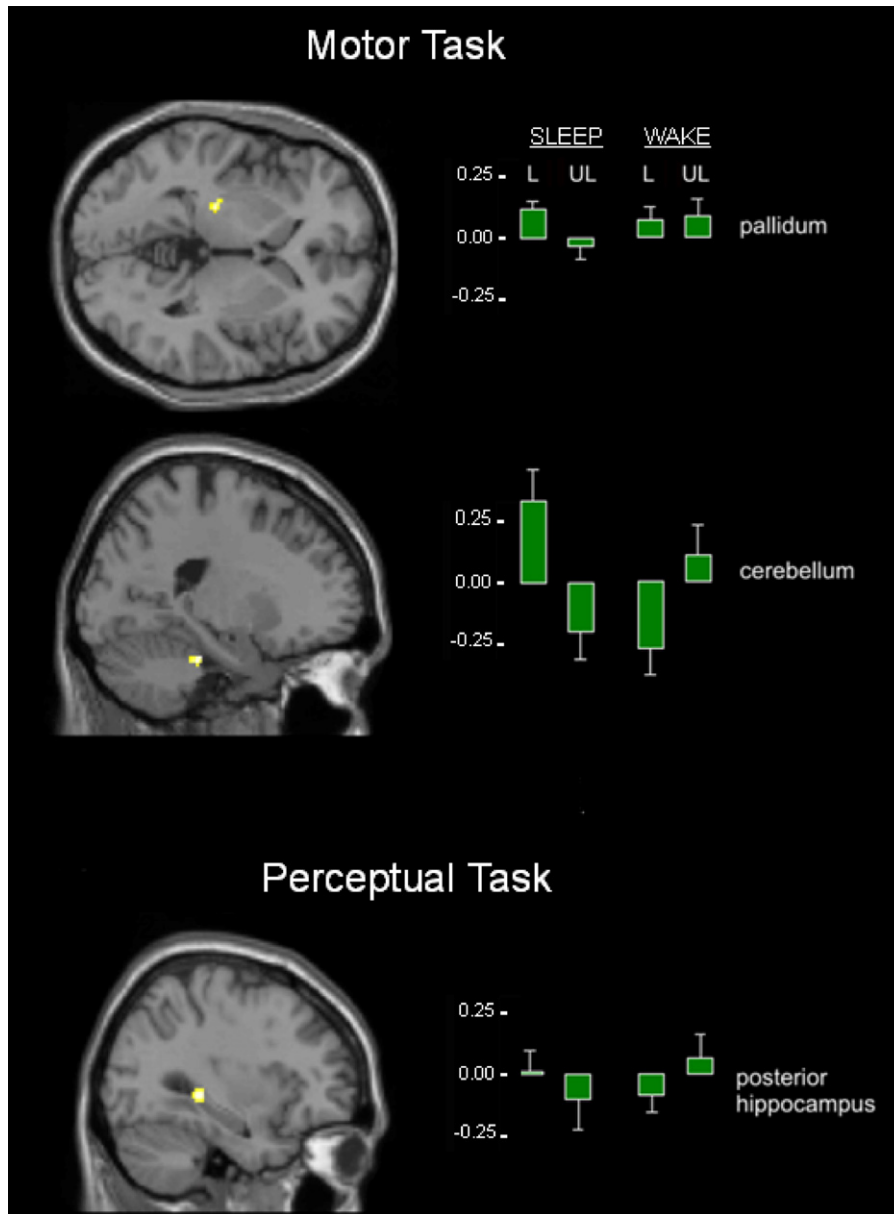


Fig. 3. Modulation of fMRI response to motor and perceptual timing by brain-state during offline retention. These results were calculated using the contrast Sleep [Learned > Unlearned] > Wake [Learned > Unlearned] for motor (Tapping) and perceptual (Monitoring) tasks. Peak parameter estimates are shown to the right (L, Learned; UL, Unlearned). Data are rendered on the SPM canonical brain at a visualisation threshold of $p < 0.005$.

Table 1
Functional responses.

Voxel count	Z	Coordinates	Anatomical region
(A) Tapping: Sleep [L-U]–Wake [L-U] (increases, contrast 1)			
7	3.6	20 –30 –28	Lateral cerebellum
5	3.5	–24 –22 0	Globus pallidus
6	3.5	10 –16 58	Medial superior frontal gyrus (SMA)
6	3.4	18 64 2	Frontal pole
(B) Tapping: Sleep [L-U]–Wake [L-U] (decreases, contrast 1)			
18	3.7	–22 –6 72	Superior frontal gyrus (PMC)
(C) Monitor: Sleep [L-U]–Wake [L-U] (increases, contrast 2)			
15	3.4	–32 –36 –2	Posterior hippocampus

MNI coordinates for the peak voxels of clusters surviving at $p = 0.001$ uncorrected and $k = 5$. Abbreviations: SMA, supplementary motor area; PMC, premotor cortex.

BOLD response during motor timing were modulated by brain state (sleep or wake) in the retention interval. This revealed peak responses in the globus pallidus of the left striatum, the SMA, and the dorso-lateral cerebellum ($p < 0.001$ uncorrected, Fig. 3 and Table 1A). In the striatum, these responses were driven largely by learning-related increases after sleep. In other regions they were driven both by learning-related increases after sleep and by learning-related decreases after wake (see parameter estimates in Fig. 3). To test for a predictive relationship between overnight improvement in behavioural performance and the overnight alterations in brain responses while performing the task, parameter estimates for the peak voxels in each response were regressed against a measure of how much performance improved after consolidation (Training CV–Scanning CV) for the Learned sequence. In the Sleep group this revealed significant correlations for both striatum ($R(12) = 0.6, p < 0.05$) and cerebellum ($R(12) = 0.57, p < 0.05$), but not for the SMA. These correlations

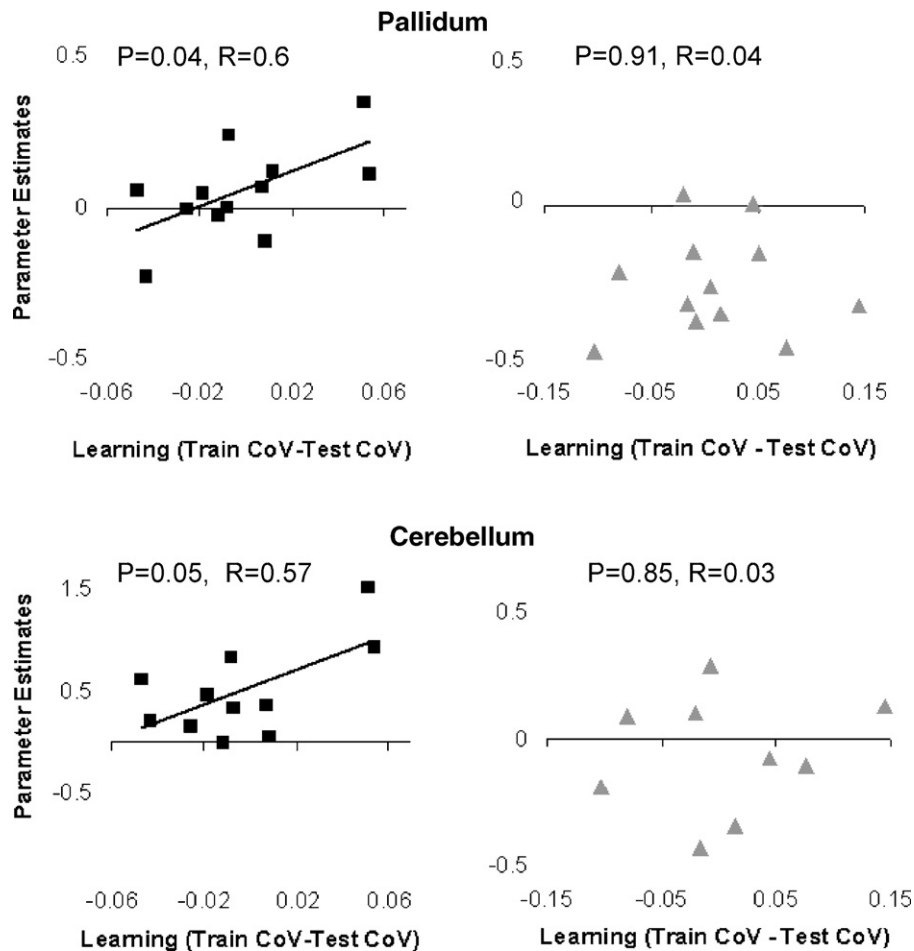


Fig. 4. Correlation between post-sleep fMRI response and performance. Responses in striatum and dorsal cerebellum correlate significantly with improvement in performance CV (Train-Test) after a delay containing sleep, but not after an equivalent delay containing wakefulness alone.

were not apparent in the wake group ($R(12)=0.04$, $p=0.91$) for striatum, and ($R(12)=.03$, $p=0.85$) for cerebellum), see (Fig. 4).

In Monitoring, the interaction analysis [Sleep(Learned > Unlearned) > Wake(Learned > Unlearned)] (contrast 2) tested for regions where learning related changes in BOLD response during perceptual timing were modulated by brain state (sleep or wake) in the retention interval. This revealed a single response in the right posterior hippocampus ($p < 0.001$ uncorrected, see Fig. 3 and Table 1C). As with the changes observed in SMA and cerebellum in contrast 1 this response was driven both by learning-related increases across sleep and by learning-related decreases across wake. The peak parameter estimate for this response correlated neither with performance of the Learned rhythm ($R(12)=0.49$, $p=0.11$) nor with differences between performance on Learned and Unlearned rhythms ($R(12)=-0.2$, $p=0.53$) in the Sleep group.

To test for a dissociation between the consolidation patterns of brain structures involved in motor and perceptual timing, results of the interaction analyses (contrasts 1 and 2 above) were compared for Tapping and Monitoring (contrast 1 <> contrast 2). This revealed greater modulation of learning-related alterations in striatal response by brain state during retention in Tapping than in Monitoring. Finally, an SPM conjunction was performed across data from Tapping and Monitoring (contrasts 1 and 2 above) and revealed no region of common activity.

Overall, our functional results show that the neural responses associated with learning a temporal rhythm differ significantly between participants who have consolidated across a night of sleep and a day of wakefulness. Furthermore, they support a dissociation

between the brain regions involved in motor and perceptual timing tasks, with components of the motor system significantly influenced by brain state (sleep or wake) during retention in the former (Tapping), while the posterior hippocampus is significantly influenced by brain state during retention in the latter (Monitoring).

4. Discussion

In this report, we demonstrate that knowledge of a temporal rhythm can consolidate overnight. We also show that brain state (sleep/wake) during twelve hours of offline retention modulates learning-related responses in parts of the motor system during motor timing, and in the posterior hippocampus during perceptual timing. These results support a dissociation between the brain systems involved in automatic and cognitively controlled timing, with the former drawing more heavily on the motor system, while the latter is more associated with processing in higher cognitive areas (Lewis & Miall, 2003).

4.1. Overnight improvements in performance

Experiment 1 demonstrates that participants can tap a complex temporal rhythm with greater consistency after a night of sleep. Good performance on this task requires both a strong representation of the temporal durations in the rhythm and a highly controlled motor output. Because our behavioural analysis did not discriminate between these two factors, the improvement we observed

could have been due to enhancements in either domain. Experiment 2 clarifies this by revealing post-sleep improvements in perceptual monitoring of the rhythm (Fig. 2C), and thus demonstrating that such consolidation is not limited to motor control but also extends to a more abstract representation of temporal sequences.

4.2. Brain state during retention modulates consolidation

Our data show that learning-related brain responses during two rhythm tasks are modulated by brain state (wake or sleep) during retention. Importantly, the location of such modulation is not the same for both motor and perceptual tasks. Instead, responses in cerebellum, striatum, and SMA are modulated in Tapping, while responses in posterior hippocampus are modulated in Monitoring. An influential model of how timed motor sequences are learned (Doyon, Penhune, & Ungerleider, 2003; Doyon et al., 2009; Penhune & Doyon, 2002; Penhune & Doyon, 2005) suggests that the cerebellum is involved in early (fast) learning, while the striatum becomes increasingly important as consolidation progresses (slow learning). Our observation of enhanced learning-related responses in both structures after sleep could therefore indicate that learning has not yet progressed beyond this early fast-stage. However, the fact that our results are driven both by BOLD increases across sleep, and by BOLD decreases across wake, makes such interpretation more complex. Specifically, while both cerebellar and striatal responses appear to increase across a night of sleep, the cerebellar response also decreases across a day of wakefulness (see Fig. 3). We speculate that while sleep is necessary to recruit the slow-learning striatal system, the quick-learning cerebellar system only disengages across daytime wakefulness, potentially in association with interference, or recruitment of this system for other tasks.

The areas where responses were modulated by brain state during retention in Tapping are all strongly associated with time measurement (Buhusi & Meck, 2005; Ivry, 1997; Macar, Vidal, & Casini, 1999). Furthermore the SMA and cerebellum have been specifically linked to motor timing (Lewis & Miall, 2003; Wiener et al., 2010), and the lateral cerebellum may be particularly important for predicting the temporal dynamics of sequential movements (Ivry, Keele, & Diener, 1988; Miall, Weir, & Stein, 1987; Miall, Weir, Wolpert, & Stein, 1993; Sakai, Ramnani, & Passingham, 2002). Although there is as yet no strong consensus regarding the precise roles of these structures in a clock mechanism (Wiener et al., 2010) for a recent review, most models agree that a clock must contain both a measurement device which marks time in a reliable way, (e.g., along the lines of a pizo-electric crystal (Gibbon, 1977), or predictably decaying function (Staddon & Higa, 1999)), and some form of memory store. Having both of these components is important because it allows for comparison of currently measured intervals against remembered targets (Bugman, 1998; Buhusi & Meck, 2005; Gibbon, 1977; Mauk & Buonomano, 2004; Staddon & Higa, 1999). As the time measurement component is by definition a stable process, it may be unreasonable to expect the brain regions performing this type of function to show an altered pattern of activity after consolidation. Instead, consolidation-related alterations in brain activity are likely associated with a modified mnemonic representation of the learned temporal durations, and may therefore relate to the memory component of the clock system.

The consolidation-related modulations of brain activity which we observed during Tapping are easy to reconcile with a memory based interpretation since both striatum (Cohen, 1984; Poldrack & Foerde, 2008; Poldrack & Packard, 2003; Poldrack et al., 2001) and cerebellum (Nixon & Passingham, 2000; Nixon & Passingham, 2001; Spencer & Ivry, 2009) are critical for motor learning, although the latter may be more important at earlier stages of consolidation (Doyon et al., 2003; Doyon et al., 2009). The modulation

observed during Monitoring is also consistent with a consolidation-related alteration in the way temporal material is remembered since hippocampal damage has been shown to alter temporal memory in rats (Meck, Church, & Olton, 1984), and these data form the basis of a model in which the hippocampus serves as a memory store for temporal durations (Meck, 2005). Furthermore, although the hippocampus plays a well established role in declarative memory (Eichenbaum, 2006; Squire & Zola-Morgan, 1991) its involvement in procedural learning, as reported here, is not unprecedented (Albouy et al., 2008; Gheysen, Van, Roggeman, Van, & Fias, 2010), and one study even demonstrated greater learning-related hippocampal responses in a procedural memory task after consolidation across sleep (Albouy et al., 2008). The neural responses reported here resulted from a combination of learning-related increases across sleep and learning-related decreases across wake, thus demonstrating that this structure's role in rhythm learning is modulated by brain state during consolidation. It is noteworthy that these responses do not indicate involvement in actual task performance, but instead index off-line consolidation. Nevertheless, the differential effects in Tapping, where we observed modulation of responses in striatum, SMA, and cerebellum, and Monitoring, where we observed modulation of responses in the posterior hippocampus, are in line with our previous suggestion (Lewis & Miall, 2003) that motor timing draws upon portions of the motor control system while nonmotor timing is more tightly associated with areas involved in cognitive processing (e.g. declarative memory and/or attention). In particular, our findings indicate that this distinction evolves off-line in a manner which is modulated by brain state (sleep or wake) during the retention interval.

4.3. Absence of overnight Tapping improvement in Experiment 2

A number of fMRI studies have reported evidence for sleep-dependent changes in memory representations that were not associated with concomitant behavioural improvements, see Sterpenich et al. (2007), Sterpenich et al. (2009), Walker, Stickgold, Alsop et al. (2005) for examples. Such findings support the widely held assumption that measurements of neural activity are more sensitive to the effects of consolidation than behavioural measures. The absence of an overnight improvement in Tapping performance in Experiment 2 raises the question of whether it is reasonable to make this same assumption in the current report. In considering this, we note that the marked overnight improvement by these same participants on the Monitoring task (Fig. 2C) demonstrates that they had access to a strengthened representation of the rhythm post-sleep. Furthermore, although we did not find an overnight improvement in Tapping performance among these participants at the group level, there was a significant correlation between the extent to which individuals improved and the functional response in both striatum and cerebellum (Fig. 4). In the context of the post-sleep Tapping enhancement observed in Experiment 1 (performed outside the fMRI scanner Fig. 2A and B), these findings suggest that the atypical environment of the scanner (e.g., the cramped supine position and loud background noise) may have impaired some participants' ability to express consolidation based memory enhancements via the Tapping task, thus precluding observation of a behavioural effect at the group level in that task.

4.4. Control for circadian effects and interference from daytime activity

Could the time of day at which testing sessions occurred provide an alternative explanation for the differences we observed between test sessions in terms of both behavioural performance and brain responses? Sessions occurring directly after retention across wake

and retention across sleep differed not only in the brain state experienced during the retention interval (asleep or awake), but also in the time of day at which they were administered (9 AM or 9 PM). It is possible that non-specific processes, such as attentional abilities are superior in the morning compared to the evening. With respect to Experiment 1, a circadian-based explanation of our findings appears unlikely due to the absence of significant inter-group differences in performance at the first session. Specifically, although the AM/PM/AM group executed their first session in the morning, while the PM/AM/PM group executed their first session in the evening, performance did not differ significantly across groups in this session ($p = 0.48$). Additionally, comparison of performance from the first and last sessions, (AM to AM and PM to PM respectively for the two groups) revealed improvements across a 24 h delay ($p < 0.02$), indicating that such enhancements were not linked to circadian test time. In Experiment 2, the impact of circadian factors upon brain response was controlled by the use of two-tiered contrasts in which Learned and Unlearned rhythms collected in interleaved blocks within the same fMRI session were compared prior to contrasts between Sleep and Wake groups. This arrangement meant that responses associated with performing the task at a specific time of day were disambiguated from those relating to the difference between consolidation across sleep and wake. In summary, both characteristics of our results in Experiment 1 and the two-tiered design in Experiment 2 help to minimize concern that differences in performance and brain response observed after consolidation across wake and sleep could have been due to circadian effects. Instead, it is parsimonious to conclude that brain state during retention may have modulated these effects. Research has suggested that sleep may play an active role in some forms of memory consolidation see Born et al. (2006), Walker (2009) for reviews, but because we did not record brain activity during sleep such speculation is beyond the scope of this paper.

5. Summary

In sum, our data indicate that knowledge of a temporal rhythm is strengthened across a period of sleep and suggest that the striatum and cerebellum serve a memory function in rhythm tapping, while the posterior hippocampus is more associated with rhythm monitoring. These findings concur with the suggestion that structures within the movement control system are recruited during motor timing, while structures associated with higher cognitive function are more involved in perceptual timing. The current report extends prior work in this area by showing that such responses are modulated by brain state (sleep/wake) during offline retention.

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References

- Albouy, G., Sterpenich, V., Baletau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., et al. (2008). Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron*, *58*, 261–272.
- Born, J., Rasch, B., & Gais, S. (2006). Sleep to remember. *Neuroscientist*, *12*, 410–424.
- Buetti, D., Walsh, V., Frith, C., & Rees, G. (2008). Different brain circuits underlie motor and perceptual representations of temporal intervals. *Journal of Cognitive Neuroscience*, *20*, 204–214.
- Bugman, G. (1998). Towards a neural model of timing. *Biosystems*, *48*, 11–19.
- Buhusi, C. V., & Meck, W. H. (2005). What makes us tick? Functional and neural mechanisms of interval timing. *Nature Reviews in the Neuroscience*, *6*, 755–765.
- Clarke, S., Ivry, R., Grinband, J., & Shimizu, N. (1996). Exploring the domain of the cerebellar timing system. In G. E. S. Vroon, & P. A. Vroon (Eds.), *Time, internal clocks, and movement* (pp. 257–280). New York: Elsevier Science Ltd.
- Cohen, D. A., Pascual-Leone, A., Press, D. Z., & Robertson, E. M. (2005). Off-line learning of motor skill memory: A double dissociation of goal and movement. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 18237–18241.
- Cohen, N. J. (1984). Preserved learning capacity in amnesia: Evidence for multiple memory systems. In L. R. Squire, & N. Butters (Eds.), *Neuropsychology of memory* (pp. 83–103). New York: Guilford.
- Dang-Vu, T., Desseilles, M., Peigneux, P., & Maquet, P. (2006). A role for sleep in brain plasticity. *Pediatric Rehabilitation*, *9*, 98–118.
- Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., et al. (2009). Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research*, *199*, 61–75.
- Doyon, J., Penhune, V., & Ungerleider, L. G. (2003). Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia*, *41*, 252–262.
- Eichenbaum, H. (2006). Remembering: Functional organization of the declarative memory system. *Current Biology*, *16*, R643–R645.
- Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2003). Consolidation during sleep of perceptual learning of spoken language. *Nature*, *425*, 614–616.
- Fischer, S., Drosopoulos, S., Tsen, J., & Born, J. (2006). Implicit learning-explicit knowing: A role for sleep in memory system interaction. *Journal of Cognitive Neuroscience*, *18*, 311–319.
- Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 11987–11991.
- Friston, K. J., Penny, W. D., & Glaser, D. E. (2005). Conjunction revisited. *Neuroimage*, *25*, 661–667.
- Gheysen, F., Van, O. F., Roggeman, C., Van, W. H., & Fias, W. (2010). Hippocampal contribution to early and later stages of implicit motor sequence learning. *Experimental Brain Research*, *202*, 795–807.
- Gibbon, J. (1977). Scalar expectancy theory and Weber's law in animal timing. *Psychological Review*, *84*, 279–325.
- Ivry, R. (1997). Cerebellar timing systems. *International Review of Neurobiology*, *41*, 555–573.
- Ivry, R. B., Keele, S. W., & Diener, H. C. (1988). Dissociation of the lateral and medial cerebellum in movement timing and movement execution. *Experimental Brain Research*, *73*, 167–180.
- Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J., & Sagi, D. (1994). Dependence on REM sleep of overnight improvement of a perceptual skill. *Science*, *265*, 679–682.
- Laureys, S., Peigneux, P., Perrin, F., & Maquet, P. (2002). Sleep and motor skill learning. *Neuron*, *35*, 5–7.
- Lewis, P. A., & Miall, R. C. (2003). Distinct systems for automatic and cognitively controlled time measurement: Evidence from neuroimaging. *Current Opinion in Neurobiology*, *13*, 250–255.
- Lewis, P. A., & Miall, R. C. (2006). Remembering the time: A continuous clock. *Trends in Cognitive Sciences*, *10*, 401–406.
- Lewis, P. A., Wing, A. M., Pope, P. A., Praamstra, P., & Miall, R. C. (2004). Brain activity correlates differentially with increasing temporal complexity of rhythms during initialisation, synchronisation, and continuation phases of paced finger tapping. *Neuropsychologia*, *42*, 1301–1312.
- Macar, F., Vidal, F., & Casini, L. (1999). The supplementary motor area in motor and sensory timing: Evidence from slow brain potential changes. *Experimental Brain Research*, *125*, 271–280.
- Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., et al. (2000). Experience-dependent changes in cerebral activation during human REM sleep. *Nature Neuroscience*, *3*, 831–836.
- Maquet, P., Schwartz, S., Passingham, R., & Frith, C. (2003). Sleep-related consolidation of a visuomotor skill: Brain mechanisms as assessed by functional magnetic resonance imaging. *Journal of Neuroscience*, *23*, 1432–1440.
- Mauk, M. D., & Buonomano, D. V. (2004). The neural basis of temporal processing. *Annual Review of Neuroscience*, *27*, 307–340.
- Meck, W. H. (2005). Neuropsychology of timing and time perception. *Brain and Cognition*, *58*, 1–8.
- Meck, W. H., Church, R. M., & Olton, D. S. (1984). Hippocampus, time, and memory. *Behavioral Neuroscience*, *98*, 3–22.
- Miall, R. C., Weir, D. J., & Stein, J. F. (1987). Visuo-motor tracking during reversible inactivation of the cerebellum. *Experimental Brain Research*, *65*, 455–464.
- Miall, R. C., Weir, D. J., Wolpert, D. M., & Stein, J. F. (1993). Is the cerebellum a smith predictor? *Journal of Motor Behavior*, *25*, 203–216.
- Nixon, P. D., & Passingham, R. E. (2000). The cerebellum and cognition: Cerebellar lesions impair sequence learning but not conditional visuomotor learning in monkeys. *Neuropsychologia*, *38*, 1054–1072.
- Nixon, P. D., & Passingham, R. E. (2001). Predicting sensory events: The role of the cerebellum in motor learning. *Experimental Brain Research*, *138*, 251–257.
- Penhune, V. B., & Doyon, J. (2002). Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. *Journal of Neuroscience*, *22*, 1397–1406.
- Penhune, V. B., & Doyon, J. (2005). Cerebellum and M1 interaction during early learning of timed motor sequences. *Neuroimage*, *26*, 801–812.
- Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, *9*, 534–547.

- Poldrack, R. A., Clark, J., Pare-Blagoev, E. J., Shohamy, D., Creso, M. J., Myers, C., et al. (2001). Interactive memory systems in the human brain. *Nature*, *414*, 546–550.
- Poldrack, R. A., & Foerde, K. (2008). Category learning and the memory systems debate. *Neuroscience and Biobehavioural Reviews*, *32*, 197–205.
- Poldrack, R. A., & Packard, M. G. (2003). Competition among multiple memory systems: Converging evidence from animal and human brain studies. *Neuropsychologia*, *41*, 245–251.
- Rammesayer, T. H. (1999). Neuropharmacological evidence for different timing mechanisms in humans. *The Quarterly Journal of Experimental Psychology B: Comparative and Physiological Psychology*, *52*, 273–286.
- Rauchs, G., Desgranges, B., Foret, J., & Eustache, F. (2005). The relationships between memory systems and sleep stages. *Journal of Sleep Research*, *14*, 123–140.
- Robertson, E. M., Pascual-Leone, A., & Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology*, *14*, 208–212.
- Sakai, K., Ramnani, N., & Passingham, R. E. (2002). Learning of sequences of finger movements and timing: Frontal lobe and action-oriented representation. *Journal of Neurophysiology*, *88*, 2035–2046.
- Spencer, R. M., & Ivry, R. B. (2009). Sequence learning is preserved in individuals with cerebellar degeneration when the movements are directly cued. *Journal of Cognitive Neuroscience*, *21*, 1302–1310.
- Spencer, R. M., Sunm, M., & Ivry, R. B. (2006). Sleep-dependent consolidation of contextual learning. *Current Biology*, *16*, 1001–1005.
- Spencer, R. M., Zelaznik, H. N., Diedrichsen, J., & Ivry, R. B. (2003). Disrupted timing of discontinuous but not continuous movements by cerebellar lesions. *Science*, *300*, 1437–1439.
- Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science*, *253*, 1380–1386.
- Staddon, J. E., & Higa, J. J. (1999). Time and memory: Towards a pacemaker-free theory of interval timing. *Journal of the Experimental Analysis of Behavior*, *71*, 215–251.
- Sterpenich, V., Albouy, G., Boly, M., Vandewalle, G., Darsaud, A., Balteau, E., et al. (2007). Sleep-related hippocampo-cortical interplay during emotional memory recollection. *PLoS Biology*, *5*, e282.
- Sterpenich, V., Albouy, G., Darsaud, A., Schmidt, C., Vandewalle, G., Dang-Vu, T., et al. (2009). Sleep promotes the neural reorganization of remote emotional memory. *Journal of Neuroscience*, *29*, 5143–5152.
- Stickgold, R., Whidbee, D., Schirmer, B., Patel, V., & Hobson, J. A. (2000). Visual discrimination task improvement: A multi-step process occurring during sleep. *Journal of Cognitive Neuroscience*, *12*, 246–254.
- Ullen, F., & Bengtsson, S. L. (2003). Independent processing of the temporal and ordinal structure of movement sequences. *Journal of Neurophysiology*, *90*, 3725–3735.
- Walker, M. P. (2009). The role of sleep in cognition and emotion. *Annals of the New York Academy of Sciences*, *1156*, 168–197.
- Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A., & Stickgold, R. (2002). Practice with sleep makes perfect: Sleep-dependent motor skill learning. *Neuron*, *35*, 205–211.
- Walker, M. P., & Stickgold, R. (2004). Sleep-dependent learning and memory consolidation. *Neuron*, *44*, 121–133.
- Walker, M. P., & Stickgold, R. (2006). Sleep, memory, and plasticity. *Annual Review of Psychology*, *57*, 139–166.
- Walker, M. P., Stickgold, R., Alsop, D., Gaab, N., & Schlaug, G. (2005). Sleep-dependent motor memory plasticity in the human brain. *Neuroscience*, *133*, 911–917.
- Walker, M. P., Stickgold, R., Jolesz, F. A., & Yoo, S. S. (2005). The functional anatomy of sleep-dependent visual skill learning. *Cerebral Cortex*, *15*, 1666–1675.
- Wiener, M., Turkeltaub, P., & Coslett, H. B. (2010). The image of time: A voxel-wise meta-analysis. *Neuroimage*, *49*, 1728–1740.
- Wing, A. M., & Kristofferson, A. B. (1973). Response delays and the timing of discrete motor responses. *Perception and Psychophysics*, *14*, 5–12.
- Zelaznik, H. N., Spencer, R. M., & Doffin, J. G. (2000). Temporal precision in tapping and circle drawing movements at preferred rates is not correlated: Further evidence against timing as a general-purpose ability. *Journal of Motor Behavior*, *32*, 193–199.
- Zelaznik, H. N., Spencer, R. M., & Ivry, R. B. (2002). Dissociation of explicit and implicit timing in repetitive tapping and drawing movements. *Journal of Experimental Psychology: Human Perception and Performance*, *28*, 575–588.