Decoupling of sleep-dependent cortical and hippocampal interactions in a neurodevelopmental model of schizophrenia.

K.G. Phillips^{1,2*}, U. Bartsch^{2*}, A.P. McCarthy^{1}, D.M. Edgar^{1}, M.D. Tricklebank^{1}, K.A. Wafford^{1}, M.W. Jones^{1,2}

^{1} Lilly Centre for Cognitive Neuroscience, Eli Lilly and Company, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK

^{2} School of Physiology and Pharmacology, MRC Centre for Synaptic Plasticity, University of Bristol, Medical Sciences Building, University Walk, Bristol, BS8 1TD, UK

*These authors contributed equally to the work.

Characters w/ spaces main text: 27,982
Characters w/ spaces legends: 4,899
Characters w/ spaces exptal procedures: 9135
Figures: 4
Tables: 0
Supplementary Figures: 4
Supplementary Methods: 1
Supplementary Table: 1
Summary: 149 words
References Main text: 51
References Methods: 0

Running title: Dyscoordination of limbic-cortical interactions
**SUMMARY**

Rhythmic neural network activity patterns are defining features of sleep, but interdependencies between limbic and cortical oscillations at different frequencies and their functional roles have not been fully resolved. This is particularly important given evidence linking abnormal sleep architecture and memory consolidation in psychiatric diseases. Using EEG, local field potential (LFP) and unit recordings in rats, we show that antero-posterior propagation of neocortical slow-waves supports clocking of hippocampal ripples and prefrontal cortical spindles during NREM sleep. This clocking is selectively disrupted in a rat neurodevelopmental model of schizophrenia: fragmented NREM sleep and impaired slow-wave propagation in the model culminate in deficient ripple-spindle coordination and disrupted spike timing, potentially as a consequence of interneuronal abnormalities reflected by reduced parvalbumin expression. These data further define the causative interrelationships between slow-wave, spindle and ripple events, indicating that sleep disturbances may lead to state-dependent decoupling of hippocampal and cortical circuits in psychiatric diseases.
HIGHLIGHTS

- Slow-wave propagation enables coordinated limbic-cortical activity during sleep
- Abnormal neurodevelopment leads to desynchronized slow waves, spindles and ripples
- Impairments reflect patterns of altered parvalbumin expression
- Interneuron dysfunction in disease may impair sleep-dependent memory consolidation
**INTRODUCTION**

Sleep supports cognitive processing, particularly through integration and organization of recently learned information into stable, consolidated memory (see Diekelmann and Born, 2010). Structured neuronal activity spanning sub-cortical and cortical regions underpins these functions, and network activity patterns during the defining neurophysiological features of REM and non-REM (NREM) sleep stages are consistently implicated in mnemonic processing. The extent to which these distinct sleep events and stages differentially contribute to dissociable cognitive processes remains unclear, but converging evidence indicates that cortical slow-waves, thalamocortical sleep spindles and hippocampal ripples during NREM sleep act in concert to preferentially support memory consolidation.

Increases in the number of slow-waves, spindles and ripples have all been correlated with overnight improvements in declarative memories in humans and spatial memory in rodents (Diekelmann and Born, 2010), consistent with these oscillations coordinating strengthening of hippocampal memory traces or their integration across neocortex during NREM sleep. The reactivation of hippocampal ensemble firing patterns occurs preferentially during CA1 sharp wave-ripples (see O’Neill et al., 2010), which are in turn temporally correlated with thalamocortical sleep spindles (Siapas and Wilson, 1998; Sirot et al., 2003; Mölle et al., 2006); spindles themselves are phase-locked to slow-waves and also associated with ensemble reactivation (Johnson et al., 2010). These temporal inter-relationships may orchestrate the induction of synaptic plasticity during sleep by aligning replay of ensemble activity associated with ripples and spindles with periods of high cortical excitability during slow-wave ‘up states’ (Diekelmann and Born, 2010). However, whilst transcranial entrainment of cortical slow-waves can improve consolidation of
hippocampal-dependent memory in humans (Marshall et al., 2006) and hippocampal ripple disruption can slow learning in rats (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010), circuit mechanisms of ripple-spindle coordination and their dependence on global sleep architecture have not been directly demonstrated.

Given the inter-dependencies between neural activity during sleep and waking behavior and the prevalence of sleep disruption in diseases including schizophrenia, depression, Parkinson’s, Alzheimer’s and Huntington’s disease, it is clear that such disruption can cause and/or exacerbate cognitive symptoms (Wulff et al., 2010). In particular, schizophrenia-associated deficits in attention and memory processing may be attributed to aberrant sleep-related consolidation mechanisms (Manoach and Stickgold, 2009) and may therefore be reflected by altered oscillatory activity patterns during sleep. Reductions in the number and power of slow-waves (Keshavan et al., 1998; Göder et al., 2006) and reductions in sleep spindle density (Ferrarelli et al., 2010; Manoach et al., 2010; Keshavan et al., 2011) have been reported in schizophrenia and correlated with either baseline cognitive deficits (Göder et al., 2006) or deficits in overnight memory recall (Göder et al., 2008; Manoach et al., 2010; Wamsley et al., 2011). Since current schizophrenia treatments are largely ineffective in alleviating cognitive symptoms, these sleep abnormalities constitute important targets for novel therapeutic intervention. However, the circuit bases of these abnormalities – in particular their links to the GABAergic dysfunction and altered oscillatory activity during wakefulness – remain unknown.

Recordings during anesthesia have shown reductions in the cortical up-state down-state transitions that underlie sleep slow oscillations in the MAM-E17 rat neurodevelopmental model of schizophrenia (Moore et al., 2006). This model
employs administration of a mitotoxin MAM (methylazoxymethanol-acetate) to pregnant rats to induce a neurodevelopmental disruption, selectively targeting limbic-cortical circuits by timing embryonic day 17 MAM injections to coincide with hippocampal and prefrontal cortical embryogenesis (Lodge and Grace, 2009). Although no single intervention can model all aspects of schizophrenia in a rodent, the MAM-E17 model is therefore particularly useful in studying limbic-cortical dysfunction in neurodevelopmental disorders.

MAM-E17 exposed rats show cognitive changes reminiscent of those seen in schizophrenia, including impairments in spatial working memory (Gourevitch et al., 2004), attentional set-shifting (Featherstone et al., 2007) and reversal learning (Moore et al., 2006). MAM-E17 exposed rats also harbor glutamatergic dysfunction (Hradetzky et al., 2011) and interneuronal dysfunction reflected by regionally-restricted reductions in parvalbumin expression (Phillips et al., 2012).

Given the evidence that disrupted sleep structure and neurophysiology are core features of schizophrenia, we hypothesized that MAM-E17 rats would show sleep abnormalities. Here, we use the MAM-E17 model to demonstrate the interdependence between sleep architecture, neocortical slow-wave propagation and ripple-spindle coordination during NREM sleep, showing that neurodevelopmental disruption can lead to impaired hippocampal-prefrontal cortical network consolidation mechanisms.
RESULTS

Fragmented NREM sleep in E17 MAM rats

We implanted 16 control and 16 E17-MAM animals with intracranial EEG electrodes over anterior motor cortex and posterior visual cortex (Figure S1), subdural EMG electrodes and telemeters; data from 14 SHAM and 13 MAM animals are presented here. Following a recovery period of 3 weeks, EEG, body temperature, locomotor activity and food and water intake were recorded continuously for a period of 144h; results are presented for the final 48h of the undisturbed recordings, allowing 96h for the animals to adapt to the procedure.

MAM-E17 rats exhibited robust circadian rhythms in all parameters measured, none of which differed significantly from SHAM controls (Figure S1). However, MAM-E17 animals did show a reduction in the total amount of NREM sleep (see Table S1 and Figure S1); this reduction was largest (9.9%) during the first 6h of the light phase (CT0-CT5; p<0.001) when the control animals slept the most (53.4±1.4% NREM per h), but remained significant during the second 6h period of the light phase (CT6-CT11, 8.3±1.0%, p<0.01) and the second 6h period of the dark phase (CT18-CT23, 6.2±0.8%, p<0.05). In contrast to the significant reduction in NREM sleep, there was no significant reduction in REM sleep (e.g. CT0-5, -1.9%, p>0.05). Time spent in each vigilance state and 2-way-ANOVA results are presented in Table S1.

A characteristic sleep deficit observed in schizophrenic patients is a decrease in sleep efficiency (time asleep/time spent in bed) due to increases in the number of awakenings during the night (sleep fragmentation; see meta-analysis in Chouinard et al., 2004). Since rodents have a polyphasic sleep cycle, we used sleep bout length as a measure of this sleep fragmentation (Figure 1). In SHAM controls, the first 1-2h of the
light phase (CT0-CT1) were associated with the longest sleep bouts (10.4±1.8min) MAM animals had a marked 48% reduction in the average NREM sleep bout length, particularly between CT0-1 (Figure1 A-B). A repeated measures ANOVA shows a significant group x time interaction average (F(3, 75)=6.7; p<0.0001, Figure1B), due to a significant reduction in NREM sleep bouts only during the light phase (CT0-6=-48%.; p<0.001, CT6-CT11=40%; p<0.01) but not during the active dark phase. The average length of the longest REM bout was similar in both SHAM and MAM animals (F(1, 25)=0.006; p=0.93, Figure1 C-D).

Since sleep abnormalities in MAM-E17 rats were consistently restricted to NREM stages, we therefore analyzed the neurophysiological features of NREM sleep in greater detail.

**NREM delta wave and spindle abnormalities in E17 MAM rats**

We first compared basic properties of the delta waves (0.3-3Hz) and spindles (10-15Hz) characteristic of NREM sleep to investigate possible underlying network mechanisms of the NREM sleep alterations in MAM treated animals.

Individual delta waves were detected over both the anterior (motor cortex M1/2) and posterior (visual cortex) EEG recording sites in MAM and SHAM animals across the entire light phase (n=8; Figures2A-B/ S2). There were no significant reductions in either the density (MAM=8.8±1.2, SHAM=8.0±1.16 waves/min) or amplitude (MAM=160±10.1, SHAM=176.5±16.5µV) of delta waves over the motor cortex. In contrast over the visual cortex we found a small change in the amplitude (MAM=156.2±21.8, SHAM=172.6±23.8µV) and a 50% reduction in the density of NREM delta waves in the MAM animals (MAM=4.7±1.2, SHAM=9.4±1.1
waves/min; p<0.05, Figure2B). Accordingly, spectral analysis of NREM sleep EEG showed a clear reduction in delta power (FigureS1).

Thalamocortical spindles were most prominent on anterior EEG electrodes (Figure2C). There was a slight but significant reduction in the density of sleep spindles during NREM sleep in MAM animals recorded over both visual (-15.1%, p<0.001) and motor cortices (-9.2%, p<0.05, Figures 2D and S2). This reduction was particularly evident in the second half of the sleep period (Group x CT, p<0.001). The reduction in spindle density recorded over motor cortex was not associated with any change in mean spindle length, amplitude or frequency (Figure2C, left panel). There was however a reduction in the amplitude of spindles recorded over visual cortex of MAM animals (-30%, p<0.01, Figure2C), although mean frequency and length remained similar to SHAM values (p>0.05, FigureS2).

120-250Hz ripple oscillations in the hippocampus are a prominent feature of NREM sleep not evident in surface EEG. We performed unilateral, dual-site medial prelimbic cortex (PrL) and dorsal CA1 tetrode recordings of local field potential (LFP) and multiple single neuron spike trains to monitor CA1 ripples and PrL spindles. Sleep recordings (1-2h) were made immediately following exploration of a maze in order to ensure delta wave- and spindle-rich sleep. Hippocampal ripple intrinsic frequencies (MAM=182±1, SHAM=179±3Hz), peak amplitudes (121±23 vs 119±18µV), lengths (36.6±1.9 vs 37.3±2.4ms) and densities (0.89±0.11 vs 0.75±0.08Hz) were normal in MAM animals (Figures 2E and S2), indicating that basic hippocampal circuitry of ripple generation was spared following E17 MAM exposure.
Altogether, mechanisms of delta wave and spindle generation in anterior/motor cortical areas appear largely intact in MAM-E17 exposed rats, as does the circuitry responsible for hippocampal ripples. In contrast, delta wave and spindle density at posterior/visual cortical sites is preferentially attenuated. We next sought to analyze temporal relationships between these network oscillations, since a number of studies have demonstrated tight coupling between slow, spindle and ripple oscillations in rodents and humans (Siapas and Wilson, 1998; Clemens et al., 2007).

**Impaired cortical delta wave synchrony**

Cortical slow oscillations during NREM sleep reflect pyramidal cell population transitions between up- and down-states. In humans, these slow waves originate more frequently in frontal regions and propagate through the cortex in an antero-posterior direction as travelling waves (Massimini et al., 2004). We therefore tested whether the reduced slow-wave density seen at posterior sites resulted from reduced antero-posterior slow-wave coordination in MAM-exposed rats.

We aligned the start times of first long NREM sleep bouts in the light phase and averaged the magnitude of Fourier coherence between motor and visual cortical electrodes across animals (Figure 3A). There was significant coherence (0.52±0.14, p<0.05) between the motor and visual cortical EEG electrodes in the SHAM animals in the 0.3-3Hz frequency range which was significantly reduced in the MAM animals (0.29±0.11; p<0.01 vs. SHAM Figure 3A). This reduction in low frequency coherence across the cortex in the MAM animals was corroborated by a reduction in the cross-correlations between motor and visual cortical delta waves between the two recording locations (Figure S3). Cross-correlations centered on the visual delta waves showed a leftward-shifted peak in the SHAM animals, indicating that on average anterior, motor
cortical delta waves precede posterior, visual cortical waves by ~50ms. This peak was significantly reduced in MAM animals, indicating a reduction in the degree of spread of delta waves across the cortex (group x time interaction  p<0.0001).

This indicates intact basic mechanisms of slow-wave generation at motor cortical sites but impaired propagation and long range coordination in the MAM-E17 model.

**Impaired clocking of spindles by delta waves**

Since delta waves are known to influence the timing of spindle oscillations, we next investigated whether the timing of thalamocortical spindles relative to delta waves was altered in MAM rats. An event triggered average of 10-15Hz bandpass-filtered EEG time-locked to the peak of the slow-wave in motor cortex shows a clear relationship between slow oscillations and spindle activity at both motor and visual sites (Figure3B). In SHAM animals, spindle power at the motor site was at a lower level in the 0.5s preceding the negative half-wave peak relative to the following 0.5s (-6.3±1.5µV vs +4.5±0.5µV; p<0.0001). 400-600 ms before the peak negativity of the slow oscillation, spindle activity decreased to an absolute minimum, followed by a strong spindle rebound covering the entire subsequent positive-going phase of the slow oscillation. This depression and subsequent rebound was even more pronounced over the visual electrode (-1.8±2.4µV vs +5.4±0.7µV; p<0.0001, Figure3B). In MAM animals, the motor cortical reduction and rebound in spindle activity around the slow-wave remained intact (-5.4±1.8µV vs +4.7±2.4µV, p<0.0001, Figure3B), whereas visual cortical spindle activity was not influenced by the timing motor cortical slow-waves (+1.4±1.2µV vs +1.6±0.78µV, p>0.05). Again these results indicate preferential disruption of network activity during NREM sleep at posterior cortical sites, likely as
a consequence of the impaired antero-posterior delta wave coordination in the MAM model.

**Disrupted ripple-spindle synchronization**

We next used CA1-PrL tetrode recordings to identify the temporal relationship between hippocampal ripples and PrL spindles. Firstly, we calculated power in the hippocampal ripple band and cortical spindle band using a 500ms sliding average window and performed cross correlation analysis on the resulting power changes over time. This revealed a robust temporal relationship between hippocampal ripple and PrL spindle power in SHAM animals: in 5 out of 6 SHAM animals, ripple power consistently peaked within 500ms prior to spindle power, whereas in MAM animals the magnitude of the correlation and the temporal coupling to the PrL spindle was greatly reduced (FigureS3). Similarly, PrL spindle-triggered averages of CA1-LFP filtered at ripple frequency (120-250Hz) showed twin peaks of ripple power in 5 of the 6 SHAM animals: the largest main peak occurred 244±67ms prior to spindles, while a second smaller peak was apparent at 849±40ms (Figure3C). In stark contrast, no peaks or troughs were observed in the MAM animals, indicating a profound decoupling of hippocampal and cortical networks during NREM (p<0.01).

To characterize finer timescales of ripple-spindle coupling, grand averages centered on individual spindle maxima were computed from hippocampal CA1 LFP filtered at ripple frequency (Figure3D). In addition to confirming that ripples tend to precede spindles in normal rats, this analysis revealed a strong modulation of CA1 ripple power by PrL spindle oscillations in SHAM animals reminiscent of the ripple-spindle relationship reported in humans (Clemens et al., 2011). Spindle modulation of
ripple power was completely lacking in some MAM-exposed animals, and on average grossly reduced in amplitude compared to SHAM animals (Figure3D).

E17-MAM exposure therefore spares the intrinsic properties of ripples and spindles, but leads to selective decoupling of ripple-spindle coordination likely to disrupt systems consolidation mechanisms.

**PrL spindle phase locking predicts PrL-CA1 unit cross correlation.**

We further used our tetrode recorded data to test whether the spike-timing of extracellularly recorded multiple single units in PrL and CA1 - particularly in relation to ongoing LFP oscillations - was affected by MAM exposure (see Wierzynski et al., 2009). Although the number of spikes fired during ripples (see FigureS4) and spindles (see below) appeared normal in MAM animals, cross-correlations between PrL and CA1 spikes occurring within 250ms time windows around the time of maximum ripple amplitude were significantly reduced in MAM animals (p<0.05, Kolmogorov-Smirnov test; Figure4A-B; see FigureS4. Although not significant, the relative timing of CA1-PrL spiking also appears shifted in MAM animals, in which there is a greater tendency for PrL spikes to precede CA1 spikes (Figure4B).

Next we analyzed PrL single unit firing during spindle oscillations. Putative PrL pyramidal cell units were classified according to spike width and firing rates (see Experimental Procedures and FigureS4) and their spiking relative to local spindle oscillations examined using phase histograms and circular statistics (see example in FigureS4). In SHAM rats, 43% of units showed firing significantly phase-locked to PrL spindles (p < 0.05, Rayleigh test of uniformity); this was more than double the proportion of phase-locked units in MAM animals (16%; p<0.01 vsSHAM, Fisher’s exact test; Figure4C), and could not be explained by differing spindle-associated spike
numbers which were near identical in SHAM (535 ±141 spikes) and MAM (568. ± 110 spikes, p=0.86) recordings. Considering only significantly phase-locked units from SHAM and MAM animals, mean circular concentration coefficients of phase-locking were lower in MAM animals (p<0.05, Figure4D), reflecting less reliable phase locking of putative pyramidal cells to ongoing spindle oscillations in MAM animals.

Combining the two unit analyses described above we show for the first time in normal animals that PrL units with the most robust spindle phase-locking fire a greater proportion of their spikes during hippocampal ripples than less spindle phase-locked units (see linear regression in Figure4E). This relationship does not hold in MAM animals: fewer PrL units are significantly phase-locked to spindles in MAM animals, but even those that are do not show any tendency to be more active during CA1 ripples. This is consistent with the reduced ripple-spindle coordination and CA1-PrL decoupling during NREM sleep in MAM rats and details novel, sleep-dependent network and single cell electrophysiological mechanisms likely to contribute to cognitive deficits in a psychiatric disease model.
DISCUSSION

Normal sleep architecture is clearly necessary for normal brain function, yet evidence linking particular neurophysiological features of sleep to particular circuit mechanisms and functional roles has only recently begun to emerge. We show that impaired antero-posterior propagation of cortical delta waves in the MAM-E17 model is associated with mis-timing of spindle oscillations during NREM sleep; hippocampal ripple events consequently fail to coordinate with spindles in directly connected neocortical regions, including PrL. The decoupling of CA1-PrL networks during NREM sleep in the MAM-E17 model is likely to reflect disrupted sleep-dependent memory consolidation mechanisms, and to model sleep abnormalities that contribute to cognitive dysfunction in diseases like schizophrenia.

Alongside sleep fragmentation, a wide range of sleep abnormalities have been reported in schizophrenia (Keshavan et al., 1990; Manoach and Stickgold, 2009), including increased sleep latency, increased wake time after sleep onset and diminished sleep efficiency (Benca et al., 1992; Chouinard et al., 2004). A number of studies confirm reductions in NREM, slow-wave sleep (SWS or N3) that correlate with measures of cognitive disorganization, impaired attention and disrupted declarative and procedural memory, indicating that impaired SWS is consistently linked to cognitive symptoms (Göder et al., 2006; Yang and Winkelman, 2006; Sarkar et al., 2010). Conversely, schizophrenic patients with mild cognitive symptoms do not show robust SWS deficits (Ferrarelli et al., 2010).

Few animal models have been examined for any of these sleep abnormalities, hence the impact of sleep disruption on the circuit basis of cognition has remained
largely unexplored. MAM-E17 exposed rats model a wide range of neuroanatomical abnormalities associated with schizophrenia (Lodge and Grace, 2009), including reduced frontal cortical thickness, increased ventricle volume (Moore et al., 2006) and a loss of prefrontal cortical and ventral hippocampal parvalbumin-expressing (PV+) interneurons (Lodge et al., 2009). Here we show that MAM-E17 rats also show a reduced amount of NREM sleep that occurs in shorter bouts than in normal animals, but no change in the occurrence of REM sleep. This fragmented sleep architecture bears a striking similarity to that seen in at least a subset of schizophrenia patients (Wulff et al., 2010), and has even been associated with increased ventricle size – also evident in E17-MAM rats – in humans (van Kammen et al., 1988). The MAM-E17 model thereby presents a unique opportunity to demonstrate links between neuropathology, sleep architecture and sleep neurophysiology.

Interplay between spontaneous synaptic inputs, intrinsic neural properties and coupled thalamocortical network oscillations generates EEG power in the 0.3-3Hz frequency range (Crunelli and Hughes, 2010). The strongly reduced delta power during NREM sleep in MAM-E17 animals could therefore arise through cortical dysfunction, altered thalamocortical input, or both. Individual delta waves of normal amplitude could still be detected in MAM-E17 rats; their numbers during NREM sleep were maintained in motor cortex EEG, but significantly reduced in EEG recorded over visual cortex (Figure 2). These data indicate that the basic circuitry of delta wave generation is intact in the MAM-E17 model. However, the reduced proportion of prelimbic cortical pyramidal cells exhibiting up-down state fluctuations seen in anesthetized MAM-E17 rats (Moore et al., 2006) might reflect an impaired ability of cortical networks to maintain, synchronize or propagate delta waves through larger areas of cortical tissue.
In humans, delta waves are initiated in frontal regions and have been described as traveling waves, propagating to posterior regions through a mesial “highway” (Massimini et al., 2004); the power, slope and coherence of delta waves are increased after learning (Mölle et al., 2004). Loss of coherence in the delta band and a significantly reduced cross-correlation between individual delta waves in MAM-E17 animals (Figure3) shows that this synchronization between cortical sites during NREM sleep is disrupted.

Loss or dysfunction of cortical PV+ interneurons, which play pivotal roles in timing pyramidal cell activity but are reduced in both post-mortem tissue from patients (Lewis et al., 2005) and in the MAM-E17 model (Lodge et al., 2009; Phillips et al., 2012), may impair the coordinated, sequential activation of intra-cortical circuits that presumably underlies slow-wave propagation. As in schizophrenia (Ferrarelli et al., 2010), MAM-17 rats also show a small reduction in sleep spindle density, which may again reflect PV+ dysfunction given the prevalence of PV+ cells in spindle-initiating reticular thalamus, plus the participation of PV+ cortical basket cells in spindle oscillations (Hartwich et al., 2009). Indeed, thalamic abnormalities are an increasingly recognized feature of schizophrenia (Adriano et al., 2010).

Our control data confirm that the onset of thalamocortical spindles precedes an increase in delta power, and that maximum spindle power coincides with the up-state of cortical slow oscillations (Mölle et al., 2006). This temporal relationship between spindles and delta waves is intact around the anterior initiation site in MAM-E17 animals and the intrinsic properties of their spindles do not differ from SHAM controls, indicating that at least some thalamocortical circuit function is maintained. However, the spindle–delta power correlation is strongly diminished over MAM-E17
posterior cortical regions, presumably as a consequence of impaired delta wave propagation. This means that posterior cortical spindles are mis-timed relative to pyramidal cell depolarization states in MAM-E17 animals, potentially attenuating the functional impact of spindle-associated firing patterns. Further evidence for mis-timing of spindle initiation in the MAM-17 model comes with the most striking result of the current study, namely the loss of synchronization between hippocampal ripples and cortical spindles (Figure 3).

The temporal coupling of hippocampal ripples and cortical spindles during NREM has been demonstrated in both rats and humans (Siapas and Wilson, 1998; Sirota et al., 2003; Mölle et al., 2006; Clemens et al., 2007), and recent human studies suggest that delta waves coordinate frontal and temporal cortical activity during sleep (Nir et al., 2011). This may arise via cortical input modulating ripple initiation (Siapas et al., 2003; Isomura et al., 2006; Mölle et al., 2006) and hippocampal spike-timing (Hahn et al., 2007). Here we extend these previous results, showing spindle phase-locking of hippocampal ripple power similar to that reported in humans (Clemens et al., 2011) in SHAM animals (Figure 3). Embedded slow-wave, spindle and ripple oscillations therefore coordinate the rhythmic firing of pyramidal cells in cortex and CA1, providing windows of opportunity for cross-structural synaptic plasticity. Indeed, oscillatory activity in both hippocampus and neocortex during NREM sleep is associated with selective re-activation of activity sequences seen during previous behaviors (O’Neill et al., 2010; Peyrache et al., 2009). The initiation of this replay through cortical delta wave-modulated input may mark the beginning of a looped circuit interaction, whereby cortical delta waves initiate hippocampal reactivation during ripples, which in turn triggers cortical reactivation during spindles (Marshall and Born, 2007).
The lack of coupling between hippocampal ripples and cortical spindles in MAM-17 rats demonstrates the crucial role of synchronized cortical slow-waves in organizing the dialogue between cortex and hippocampus by providing a temporal framework for faster oscillations. Disrupting this dialogue presumably constitutes the neurophysiological mechanism for behavioral deficits in long term learning and memory described in the MAM E17 model (Flagstad et al., 2004; Gourevitch et al., 2004; Moore et al., 2006), and may contribute to cognitive deficits in other models of sleep fragmentation (Tartar et al., 2006).

To conclude, our study serves to emphasize the fact that specific aspects of cognitive processing continue during sleep, and that disrupted thalamic-cortical-limbic network activity during sleep must therefore be considered alongside waking activity as a therapeutic target in both schizophrenia and related diseases. Since active entrainment of slow-waves through trans-cranial stimulation enhances both spindle density and declarative memory in humans (Marshall et al., 2006) one intuitive possibility would be to use transcranial stimulation as a possible therapy for relieving cognitive and sleep deficits found in patients. The MAM-E17 model provides a unique opportunity to study the detailed cellular, synaptic and network mechanisms that underpin such novel therapeutic approaches.
ACKNOWLEDGEMENTS
We thank L. Appel, S. Shahabi, E. Shanks, for help with surgery, W. Seidel and D. Kellett for advice and support in the analysis of sleep recordings and D. Ford for assistance with histology. We would also like to thank J.T. Isaac and S.W. Hughes for critical reading of the manuscript. This work was supported by the Lilly Centre for Cognitive Neuroscience, Eli Lilly and Company, UK and the Medical Research Council (UK) grant number G0501146.

AUTHOR CONTRIBUTIONS
K.G.P. designed and performed all experiments, analyzed data and wrote the manuscript. U.B. designed and performed the tetrode recordings, wrote custom analysis routines and wrote the manuscript. A.P. scored the sleep recordings and analyzed data. D.M.E and M.T provided design and conceptual advice, technical support, and contributed to the manuscript. K.A.W and M.W.J. were responsible for the original ideas, experimental design, data analysis and editing the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

CORRESPONDING AUTHOR
Correspondence to: Matt Jones (matt.jones@bristol.ac.uk)
**FIGURE LEGENDS**

**Figure 1**

*Fragmented NREM sleep in E17-MAM exposed rats*

(A) Timecourse of average NREM sleep bout length over a 48h undisturbed recording period. Data are plotted as mean ± S.E.M. with light/dark bars along the x-axis indicating lights on/off. Note the longer sleep bouts at the beginning of the lights on (CT0-2) in SHAM (grey) which are reduced in the MAM animals (blue). (B) Bars indicate mean NREM sleep bout length over a 24h period averaged into 6 hourly bins. ANOVA shows significant group x time interaction for average sleep bout length ($F(3,75)=6.7; p<0.0001$). (C-D) Equivalent time course and analysis of average REM bout lengths over the same 48h period ($F_{(1,25)}=0.006; p = 0.93$). Differences between the SHAM (n=14) and MAM (n=13) groups compared using a Bonferroni corrected t-test are indicated by asterisks (**p<0.001, *p < 0.01, * = p<0.05).*

**Figure 2**

*Selective delta wave and spindles abnormalities during NREM sleep in E17-MAM rats*

(A) Event triggered averages of raw EEG centered on the negative peaks of the delta waves from motor (left) and visual cortex (right) in SHAM (n=14, black) and MAM (n=13, blue) animals. The amplitudes and slopes of delta waves are similar in both groups. (B) Delta wave densities from the motor (left) and visual (right) cortical EEG electrodes in SHAM (grey) and MAM (blue) animals. Delta wave density is significantly reduced over the visual cortex in the MAM animals (group x time interaction $F_{(1,25)}=5.2; p < 0.05$). Data are taken from the first 2h of the animals’ sleep
period when slow-wave power is highest. (C-D) Average spindle waveforms (C) and densities (D) recorded over motor (left) and visual (right) cortex showing more marked reduction of spindle density at posterior (-15.1%, t(14)=6.3, p<0.001 vs SHAM) relative to anterior (-9.2%, t(14)=2.6, p<0.05 vs SHAM) sites in MAM animals during late sleep epochs. (E-F) CA1 ripple waveforms and densities appear normal in MAM animals.

**Figure 3**

**Delta waves, spindles and ripples are desynchronized in MAM animals**

(A) Multitaper Fourier coherence between motor and visual cortical EEG during NREM sleep (bandwidth 0.2Hz, 10s moving window, 4 tapers) is significant in the 0.3-3Hz frequency range in SHAM animals (black, n=8, p<0.05) and significantly reduced in the MAM animals (blue, n=8, p<0.01 vs SHAM), indicating impaired anterior-posterior delta wave coordination. (B) Delta wave-spindle coordination is also disrupted in MAM animals. Motor delta wave- (dotted line shows average delta waveform) triggered spindle power shows similar patterns at anterior sites in SHAM and MAM animals (upper graph) but is severely disrupted at posterior cortical sites (lower graph) in MAM animals. Note the characteristic occurrence of spindles on the rising phase of delta waves in SHAM animals. (C) PrL spindle-triggered averaging of CA1 ripple power (from tetrode recordings of LFP, 10ms bin width) shows that ripples consistently precede spindles in SHAM animals (black, n=6) but that this timing relationship is abolished in MAM animals (blue, n=5; Bonferroni corrected pair-wise comparison p<0.05). (D) Alignment of average PrL spindle waveforms (upper graph) with ripple band RMS activity shows fine timescale, spindle-frequency
modulation of CA1 ripples in SHAM animals (middle graph) and its attenuation in MAM animals (lower graph).

**Figure 4**

**Attenuated CA1-PrL unit cross-correlations and spindle phase-locking in E17-MAM rats**

(A) Left-shifted cumulative distribution function of peak cross-correlation values between CA1 and PrL putative pyramidal cell spike times during ripples in MAM animals (blue) relative to SHAM controls (black). (B) Mean ± S.E.M. normalized cross-correlations between CA1 (Time lag=0s) and PrL units for SHAM (black, N=4, n=232) and MAM (blue, N=4, n=332) animals showing a significantly reduced short-latency peak in MAM animals (p<0.05, Kolmogorov-Smirnov test). Lower panels plot distributions of CA1-PrL cross-correlation peak times; note left-shifted peak indicating that PrL units tend to fire before CA1 units during ripples in MAM animals. (C) The overall percentage of significantly phase locked putative pyramidal cell units in PrL is higher in SHAM animals (38%, total N units=38) compared to MAM animals (15%, total N units=46). Fisher’s exact test for binomial distributions indicates a significant difference in the percentages (p=0.007). (D) Mean circular concentration coefficient (kappa) values for all significantly phase locked PrL units in SHAM (black) and MAM (blue) animals (p=0.029, t-test). (E) Regression analysis showing that the extent of PrL spindle phase-locking (Rayleigh kappa) correlates with the proportion of spikes fired during CA1 ripples for PrL putative pyramidal cells in SHAM animals (black, R²=0.72, p<0.0001) but not MAM animals (blue, R²=0.1, p>0.5). Note that the correlation holds even without including the outlying SHAM unit with kappa = 0.7.
**EXPERIMENTAL PROCEDURES**

**Subjects**
All procedures were carried out in accordance with the UK Animals Scientific Procedures Act 1986. Timed pregnant Sprague–Dawley dams were obtained from Charles River (UK) on day 12 of gestation. On E17 rats were injected intraperitoneally with saline or MAM (22 mg/kg, in a volume of 1 mg/ml; Midwest Research Institute, Kansas City, Missouri). In total, 15 dams were injected with saline and 15 dams were injected with MAM producing 49 MAM pups and 51 SHAM pups. No more than two animals used were derived from a single litter.

**EEG recordings**
Between 70 and 80 days of age rats were anesthetized with isoflurane and prepared with a cranial implant consisting of five stainless steel screws, two located over motor cortex at +3.9mm AP, ± 2.0mm ML from bregma, two visual cortex −6.4mm AP, ±5.5mm ML from bregma, and one ground screw over the cerebellum. EMG was monitored via two Teflon-coated stainless steel wires positioned under the nuchal trapezoid muscles. Body temperature (Tb) and locomotor activity (LMA) were monitored via miniature transmitters (PDT-4000 E-Mitter, Minimitsiter, Bend, OR) implanted in the abdomen.

Sleep/wake parameters were monitored using “SCORE-2004”, an updated version of a real-time sleep/wake and physiological data collection system previously described (Van Gelder et al., 1991). Bipolar differential EEG recordings were amplified 10,000 times, filtered (band pass, 0.3–30 Hz,) digitized at 100 Hz. EMG was amplified filtered (band pass, 10–100 Hz), then digitally integrated (root mean square) and used to further discriminate arousal states. In parallel with the differential EEG recording
used for the sleep scoring, EEG signals from the contralateral motor and visual cortices were recorded and digitized at 2kHz (CED1401, Cambridge Electronic Design, UK) for EEG frequency analyses. These separate EEG recording were referenced to neutral ground screw located over the cerebellum. Drinking and feeding activity were detected via infrared beam-break. All variables were monitored continuously and simultaneously. Arousal states were classified as awake, NREM sleep, REM sleep (REM), or Theta dominated wake (TDW) every 10s. Arousal states were determined using the automated sleep scoring algorithm based on SCORE™ that uses a combination of the salient features of the EEG and muscle tone; see Van Gelder et al., 1991 and supplementary methods for more detail. Bout length was defined as continual episodes of NREM/REM not interrupted by two or more consecutive 10s epochs of WAKE. All bouts commencing in a given hour were averaged and expressed as minutes per hour.

**Tetrode Recordings**

Six SHAM animals and five MAM animals were implanted with arrays of adjustable tetrode recording electrodes targeted to the PrL (+3.2 mm, +0.6 mm from bregma) and ipsilateral dorsal CA1 (−3.6 mm, +2.2 mm). Continuous LFP (sampled at 1KHz per channel, band pass filtered between 0.01 and 475 Hz) and single unit activity (sampled at 32.5 kHz, bandpass filtered at 600Hz-6kHz) were recorded using a Digital Lynx recording system (Neuralynx, Bozeman, MT). Local reference electrodes were placed in a proximal cortical region without spiking activity (1–2 mm below the pial surface for PrL recordings) or in overlying white matter (CA1). Positioning in white matter was achieved on the basis of characteristically flat LFP recordings with no hippocampal sharp waves or ripples. A ground screw was also
placed over the cerebellum. Electrolytic lesions established tetrode positions at the end of each experiment (Figure S4).

Sleep recordings were made immediately following exploration of a linear maze in order to ensure slow-wave- and spindle-rich sleep. Spectrograms were generated from hippocampal and PrL electrodes for the full sleep period; NREM spindle rich sleep periods were easily identifiable by clear changes in the LFP to strong power in the spindle (10-15Hz) and delta wave (0.3-3Hz) frequency ranges. A spindle power detection algorithm (see below) in combination with automated scoring of immobility from video allowed “pure” sleep periods to be identified and concatenated together. Only the first 10min of spindle rich sleep was used for the analysis to limit the sleep period to within the first hour after exploration of the maze.

**Detection algorithms and spectral analysis**

We implemented custom MATLAB (The MathWorks, Inc., Natick, MA) routines for the detection of individual EEG or LFP oscillatory sleep events (cortical delta wave, cortical spindle, hippocampal ripple). All EEG or LFP traces were first band pass filtered (slow-waves: 0.3-3 Hz, spindles: 8-18 Hz, ripples: 120-250 Hz; least squares filtering, EEGlab toolbox, Delorme and Makeig, 2004) and then transformed to z-scores.

Delta waves were detected directly from the filtered, z-score signal with a threshold of 3.5 times the SD of the whole signal in both negative and positive direction. Only waves that contained a negativity larger than 80 µV were included. The criteria for delta wave detection were applied independently to both motor and visual cortical electrodes, used for all animals and resulted in a reproducible and specific detection of high-amplitude slow-waves that occurred during late sleep in each bout.
For detection of cortical spindles and hippocampal ripples the z-score of the respective filtered signal was rectified and the envelope was determined by using a cubic spline data interpolation between the maxima of the rectified signal. This envelope was then used for detection of threshold crossings. Spindles were detected using a threshold of 3.5 x SD of the signal. Start and finish times of the spindle were also calculated (2 x SD of the signal). Spindles were rejected if they were shorter than 500ms or longer than 2s. If two spindles occurred within 100ms they were treated as one event. Ripples were detected using a threshold of 3.5 x SD of the signal. Start and finish times of the spindle were also calculated (2 x SD of the signal). Ripples were rejected if they were shorter than 50ms or longer than 500ms. If gaps of less than 50ms occurred between ripples, they were treated as one event. All automatic detection procedures were validated and its parameters were tuned through independent visual scoring of the EEG/LFP.

Multi-taper spectral analysis was used to calculate power spectra and coherence for LFP data between the two cortical recording locations (Chronux toolbox, www.chronux.org). To investigate the temporal relationship between hippocampal ripples and spindle events on a slow time scale, cross-correlation between the respective powers was computed. Spectral power in these frequency bands was calculated in overlapping 1-s windows, providing a time series, which were log-transformed and cross-correlated.

**Unit clustering and putative pyramidal cell identification**

Raw threshold detected spikes were clustered using a combination of KlustaKwik (K.D. Harris, http://klustakwik.sourceforge.net/) and manual clustering with MClust (A.D. Redish, http://redishlab.neuroscience.umn.edu) in Matlab. We used spike
energy, peak position and the first principal component of the waveform for clustering. Occasionally noise spikes were removed based on waveform features (waveform cutter). This yielded 100 units in PrL and 54 units in CA1 from 4 SHAM animals; and 70 units PrL and 44 units in CA1 from 4 MAM animals. Putative pyramidal cells were well-separated units which had a mean firing rate during nREM between 0.05-1 Hz, and with a spike width larger than 234µs (FigureS4).

For spindle phase-locking analyses, a continuous phase was calculated from the Hilbert transform of one PrL LFP trace (band passed at 8-18Hz, least squares filter, EEGLab toolbox) and individual spike phase values were calculated by local linear interpolation of spindle phase for each spike time. Only spikes within ±5s of spindle peaks and troughs larger than ±3.5 times the standard deviation of the entire signal were included. We used Rayleigh’s test to assess a significant deviation from a uniform circular distribution of spike timing and calculated the circular concentration coefficient kappa to quantify the strength of unit phase locking.

NREM cross-correlation analyses were adapted from Wierzynski et al. (2009) and are summarized in FigureS4. Putative pyramidal cell spike times from CA1 and PrL were first filtered to select spikes that occurred within ±250ms of CA1 ripples; only units that fired at least 100 spikes during ripples were included. Cross-correlations were then calculated with 10ms bin size, and normalized by conversion to z-scores before peak cross-correlation values and time lags were detected.

**Statistical analysis**

Unless otherwise stated data is expressed as mean ± S.E.M. T-tests or repeated measure ANOVAs were used to test for significant differences between MAM and SHAM (identified in results). Any significant effects that were identified in the
ANOVA were then investigated further using a Bonferroni t-test to correct for multiple comparisons. Normality of the data was checked using D'Agostino & Pearson omnibus normality test (Graphpad software).
REFERENCES


causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. Neuropsychopharmacology 29, 2052–2064.


34
Figure 1

A. Average NREM sleep bout

B. NREM sleep bout (min)

C. Average REM sleep bout

D. REM sleep bout (min)

Circadian Time

Circadian Time

0 - 5
6 - 11
12 - 17
18 - 23
Figure 2

A Delta Waves
Motor cortex EEG (0.3-3Hz) Visual cortex EEG (0.3-3Hz)

B Delta wave density (number of waves per minute)
Motor Visual
SHAM MAM

C Spindles
Motor cortex EEG (10-15Hz) Visual cortex EEG (10-15Hz)

D Spindle density (number of spindles per minute)
CT 0 - 5 CT 6 - 11 CT 0 - 5 CT 6 - 11
Circadian Time

E Ripples
CA1 LFP (120-250Hz)

F Ripple density (mg)
SHAM MAM
Figure 3
Figure 4
Supplementary Figure S1

Time course of (A) locomotor activity, (B) body temperature and (C) NREM sleep expressed as % NREM per hour over a 48h undisturbed period; note the peaks in NREM sleep at the beginning of the lights on phase (CTO-2). Repeated measures ANOVA indicates a significant group \((F_{(1,25)}=16.4; p < 0.001)\) and time effect \((F_{(3,25)}=261.4 p < 0.0001)\) (D) REM sleep, expressed as % REM sleep per h over a 48 hour period. Bars indicate data averaged into 6h bins. (E) Schematic diagram to indicate location of EEG recordings with example EEG taken from a SHAM animal. (F,G) 10s example visual cortical EEG trace, overlaid on the corresponding spectrogram taken 30s before the end of the first long sleep bout in the light phase (CT0). (F) Representative example taken from a SHAM animal, (G) is taken from the equivalent time point in the sleep bout from a MAM animal.* denote delta waves. (H) Time course of mean delta power (0.3-3Hz) ± SEM in NREM epochs from visual-motor differential EEG, expressed as µV^2 and averaged into hourly bins, over a 48h period. Black line = SHAM, blue line = MAM treated rats; shaded area indicates SEM. Bars indicate delta power in NREM epochs over a 24 hour period averaged into 6 hourly bins. Repeated measures ANOVA shows a significant group \((F_{(1,25)}=8.1; p < 0.01)\) and group x time interaction \((F_{(3,75)}=4.5; p < 0.01)\). (I) Delta power (0.3-3Hz) over time from the start of aligned sleep bouts. SHAM animals have steep increases in delta power initially that continues to rise more slowly throughout the sleep bout. MAM animals have a significantly shallower gradient in the slow-wave power rise \((p < 0.001)\). Data plotted as mean ± S.E.M. and light/dark bars along the x-axis indicate lights on/off. In all cases significant differences between the SHAM and MAM groups compared using a Bonferroni corrected t-test are indicated by an asterisk at the top of the plots (** = p < 0.001, * = p < 0.05). Data were obtained from 14 SHAM and 13 MAM-treated rats.
Supplementary Figure S2

(A) Delta wave properties (A1) Grand averages of delta wave triggered EEG recorded over visual (left) and motor cortices (right) from SHAM (black line) and MAM animals (blue line n=8 per group). Average histograms of all delta waves detected from SHAM (black) and MAM (blue) showing the distribution of delta wave amplitudes (A2) lengths (A3) and frequencies (A4) between the two groups. Dotted lines in A2 indicate the threshold for the detection algorithm. (B) Spindle properties (B1) Grand averages of spindle triggered EEG recorded over visual (left) and motor cortices (right) from SHAM and MAM animals. Average histograms of all spindles detected between CT0-11 from MAM (black) and SHAM (blue) showing the distribution of spindle amplitudes (B2) lengths (B3) and frequencies (B4) between the two groups. (C) Ripple properties (C1) Grand averages of ripple triggered 140-240Hz filtered CA1 depth LFP recorded from SHAM (black line) and MAM (blue line) animals. Average histograms of all ripples detected from MAM (black) and SHAM (blue) showing the distribution of ripple amplitude (C2) length (C3) and frequency (C4) between the two groups. (D) Peri-event time histograms (PETH) showing similar firing of CA1 putative pyramidal cells during ripples in SHAM and MAM animals. Upper panels show mean, normalized PETHs centred on ripple maxima (Time lag = 0) for 33 SHAM (left, N=4) and 34 MAM (right, N=4) CA1 units included according to the firing rate criteria used by Wierzynski et al. (2009). Shaded area shows mean±sem. Lower panels show color-scaled PETHs for all individual units.
Supplementary Figure S3

(A1) Average multi taper coherogram plot, showing coherence between motor and visual EEG recording sites during NREM sleep at different frequencies over time. Sleep bout onset times in the light phase were aligned across animals in SHAM (top) and MAM (bottom) to allow coherence within a sleep bout to be averaged. The bandwidth was set at 0.2Hz and a 10sec moving window was used. The SHAM animals show high levels of coherence in the 0.3-2Hz frequency range, which is reduced in the MAM animals. (A2) Motor visual slow-wave cross correlation referenced to visual cortical slow-waves. The peak in the cross correlation is offset to the left indicating that on average anterior slow-waves precede slow-waves in the posterior, visual cortex. The synchrony of cortical slow-waves is reduced in the MAM animals as shown by the reduction in the cross correlation peak (group x time interaction $F_{(37, 444)}=4.3; p < 0.0001$). Data are normalized to the total number of slow-waves detected during the recording period. (B) Examples of a cross-correlogram of spindle power to ripple power in a single control SHAM (B1) and MAM animal (B2). The clear peak in the ripple spindle cross-correlogram at ~300ms in the SHAM animal is clearly shifted and reduced in the MAM animal. (B3) Group data (SHAM n= 6, MAM n= 5) showing a significant reduction in the temporal correlation of ripple-spindle times in the MAM animals (blue line) compared to control SHAM animals (black line) (group x time interaction $F_{(1, 59)}=2.1; p < 0.001$).
Supplementary Figure S4

(A) Example histological verification of tetrode positions for both SHAM (A1, A2) and MAM (A3, A4) animals in the mPFC and dorsal CA1. Black arrows indicate post-mortem lesions. (B) CA1–PrL spike train cross-correlation analysis. Example raw data show wide-band CA1 (upper trace, green; sharpwave-ripples marked by arrowheads) and PrL (lower trace, black) LFP during NREM sleep. Rasters show examples of single CA1 and PrL units, exemplifying firing patterns during ripples and spindles. (C1) Histogram of spike widths (peak to trough) of all recorded PrL units. A bimodal Gaussian probability density function (PDF, grey line) was fitted to the histogram, with \( \mu_1 = 177.05 \mu s \) and \( \sigma_1 = 16.64 \mu s \) for the narrow spike width population, and \( \mu_2 = 301.48 \mu s \) and \( \sigma_2 = 33.95 \mu s \) for the wide spike width population (putative pyramidal cells). The cut-off for putative pyramidal cells was set to \( \mu_2 - 2\sigma_2 = 234 \mu s \), as indicated by the red line. (C2) Scatter plot of spike width against NREM firing rate,
showing the 1Hz maximum firing threshold for putative pyramidal cells (dotted red line; Wierzynski et al. 2009). (D1) Cross-correlogram for the example units shown in B (bin size 10ms), using all spikes during a single recording session in the sleep box (red lines mark mean firing rate and 95% confidence estimates; black lines 100ms). (D2) Cross-correlogram for same cell pair using only spikes fired within 250ms of ripple peaks during NREM sleep.

**Supplementary Table S1**

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<td>(from lights on)</td>
<td>CT0</td>
<td>ON</td>
<td>75.4 ± 8.0</td>
<td>14</td>
<td>81.6 ± 15.2</td>
<td>13 6.2</td>
<td>2.40</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
Supplementary Methods

**EEG recording environment**

Rats were housed individually within microisolator cages equipped with a custom low-torque slip-ring commutator (Hypnion, Inc., Lexington, MA), and hardware that permitted *ad libitum* access to food and water. Each Cage was located within a separate compartment of an electromagnetically shielded, ventilated, 16-animal stainless steel sleep recording chamber. Rats’ circadian rhythms were entrained to a 24-h (LD 12:12) light-dark cycle (30–35 lux inside the cage).

**Sleep scoring**

Arousal states were determined using the automated sleep scoring algorithm based on SCORE™ that uses a combination of the salient features of the electroencephalogram and muscle tone see Van Gelder et al., 1991. The process of assigning a state of wakefulness, TDW, REM or NREM sleep consisted firstly of extracting four features from the 10 second EEG epoch: amplitude, zero-crossings (a measure of EEG frequency), harmonic amplitude and harmonic frequency, these were then represented as 12 bin histograms. A mean of the integrated EMG over the 10s epoch along with the Boolean data from the LMA, drinking and feeding activity were also used in arousal state assignment. Unique templates were then generated from these parameters through a user-dependent teaching process; all users were blinded to treatment. The state scoring procedure was then performed using a pattern matching algorithm (explained in detail in Van Gelder et al., 1991), after exclusion of particular states on the basis of nonEEG data. REM sleep and NREM sleep for example, were excluded from consideration if the animals had moved or been drinking from its bottle during the epoch.

For analysis purposes, Wake and TDW were combined (WAKE). Data quality was assured by frequent on-line inspection of the signals. Sleep-wake scoring was scrutinized carefully for artefact by off-line visual examination of raw EEG waveforms and the distribution of integrated EMG values. EEG containing artefact was not used in EEG frequency analyses. All off line sleep analysis was performed blind with respect to MAM/SHAM treatment.