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Mechanisms Underlying State Dependent Surface Evoked Response Patterns

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Abstract

Cortical evoked response potentials (ERPs) display a rich set of waveforms that are both context and state dependent. However, the mechanisms that underlie state dependent ERP patterns are unclear. Determining those mechanisms through analysis of single trial ERP waveform signatures may provide insight into the regulation of cortical column state and the roles that sleep plays in cortical function. We implanted rats with EEG and EMG electrodes to record ERPs and to assess sleep/wake states continuously during 1-2s random auditory clicks. Individual cortical auditory ERPs were sorted into one of eight behavioral states, and fell into three categories based on amplitude and latency characteristics. ERPs within waking and rapid eye movement (REM) sleep were predominately low amplitude and short latency. Approximately 50% of ERPs during light quiet sleep (QS1 and QS2) exhibited low amplitude, short latency responses, and the remaining ERPs had high amplitude, long latency responses. This distribution was characteristic of EEG fluctuations during low frequency delta waves. Significantly more individual ERPs showed very low amplitudes during deep quiet sleep (QS3 and QS4), resulting in a lower average ERP. These results support the hypothesis that evoked response amplitudes and waveform patterns follow specific EEG patterns. Since evoked response characteristics distribute differently across states, they could aid our understanding of sleep mechanisms through state related and local neural signaling.

Keywords

Auditory; Rat; Quiet sleep; Slow-wave sleep; Delta

Introduction

Cortical evoked response potential (ERP) waveforms reveal specific amplitude and latency characteristics related to sleep and waking states (Weitzman and Kremen 1965; Hall and Borbely, 1970; Mendel and Goldstein 1971; Howe and Serman 1973; Arnaud et al. 1979; Pena et al. 1999; Rector et al. 2005; reviewed in Colrain and Campbell, 2007). State dependent ERPs exhibit similar characteristics irrespective of sensory modality and species for all mammals tested. Specifically, the ERP amplitude is significantly higher during quiet sleep when compared to both waking and REM sleep, and state dependent ERPs might be used to

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assess levels of sensory information processing, during both wake and sleep (reviewed in Colrain and Campbell, 2007; Coenen and Drinkenburg, 2002). The physiological mechanisms responsible for higher-amplitude ERPs during sleep are unclear, and may provide insights into roles that sleep plays in cortical function.

To characterize single trial ERPs, we used a basis function fitting routine to extract response features that occurred after each stimulus. Individual ERPs are highly variable, and EEG amplitude fluctuations are larger than ERPs, making identification of ERP components difficult without signal averaging. Earlier ERP studies concluded that much of the variability resulted from ongoing cortical activity (Arieli et al. 1996; Azouz and Gray 1999); however, others showed that the variability may result from transitions in thalamo-cortical excitability (Kisley and Gerstein 1999; Steriade et al. 1993, 2001). Specifically, ERP amplitude fluctuations during slow wave sleep may be directly related to the phase of slow waves (Massimini et al., 2003). Since quiet sleep is characterized by delta (0.1 to 4Hz) rhythms generated by slow thalamocortical oscillations with roughly a 50% duty cycle, cortical cells should generally spend half their time in a hyperpolarized state, and the other half in a depolarized state. Thus, if ERP amplitude is related to membrane potential, we predict single trial ERP analysis during quiet sleep will exhibit half high amplitude, long latency responses, and half reduced-amplitude, short latency responses. Both experimental and modeling studies suggest that cells within a cortical column are strongly coupled (e.g. Traub et al., 2005), and could synchronously enter their hyperpolarized and depolarized states at any time (Rector et al., 2005), thus the characteristics of individual ERPs could serve as a valuable probe into the processes that regulate these states.

Determining the behavioral state for each ERP is particularly difficult in rodents that exhibit short sleep cycles. Such animals exhibit multiple state transitions which are not usually divided into separate quiet sleep stages, as in human sleep, since the animals exhibit more-subtle differences in EEG delta and EMG power. Several investigators have identified two wake, three quiet sleep, and two REM sleep stages in rodents (Arnaud et al. 1979; Gottesmann 1992). However the functional significance of these sub-states is not fully characterized. Additionally, EEG recordings from rodents show many state transitions lasting only a few seconds and sleep defined in long intervals (10 s in rat, and 30 s in human) is arbitrary, chosen for ease of scoring, not for physiological reasons. Rodents can commonly be asleep, then wake for a couple seconds, then be asleep again for the next few seconds. Short sleep stages are often ignored, and the stage is taken as the dominant state during a 10 s period, or ignored all together. For human sleep, short transitions are not as common under normal conditions, but occur more often under pathological conditions. Regardless, when brief EEG identified arousals occur, they are associated with awake mentation (Steriade, 2000).

To provide the sensitivity necessary to differentiate sub-states within quiet sleep, we used cluster analysis procedures typically used to sort units from multi-unit recordings. In cluster analysis for sleep staging, different parameters from successive sleep epochs were displayed on an X-Y plot, forming clusters of common characteristics (Friedman and Jones 1984; Drewes et al. 1995; Veasey et al. 2000; Turakhia et al. 2003). Sleep scoring was assisted by smoothing the scattergram image with contour techniques to statistically define clusters. Each epoch was then visually scored using EEG, EMG and EKG to confirm the staging of each bin within the clusters.

We hypothesize that average ERPs are larger and delayed during quiet sleep due to an increased number of large individual ERPs, possibly because cortical cells spend about half of the time in the hyperpolarized condition. Thus, single trial ERP signatures may be used to assess the aggregate membrane potential of cortical cells, and could provide a real-time, non-invasive probe of cortical column physiological state and cellular signaling during sleep and sensory

processing. Since cortical information processing and task performance may be compromised when cortical column cells are hyperpolarized, these results provide an important foundation for future studies of sleep and human performance (Colrain and Campbell, 2007).

Methods

Surgical procedures

Electrodes for sleep physiology were implanted into ten Sprague Dawley rats (5 male and 5 female) under Ketamine/Xylazine (100 mg/kg and 5 mg/kg, respectively) anesthesia. Two insulated and multi-stranded stainless steel wires (Cooner Wire, Chatsworth, CA) were placed subcutaneously along either side of the thoracic cavity to record respiration and EKG. A 2 mm section of the insulation was removed at the end of the wires to detect electrical signals. Two similar wires were sewn into the neck musculature to record muscle tone (EMG), and 6 skull screw electrodes were inserted into 0.9 mm burr holes for cortical EEG recording: one frontal, and two pairs on each side over the parietal lobes, and a reference screw over the occipital bone. All wires were directed to the top of the head where a dental acrylic mounting stage was constructed to hold a connector. All animal procedures were approved by the Washington State University Animal Care and Use Committee.

Recording Procedures

One week after surgery, electrodes were connected to physiological amplifiers through a thin (4mm diameter) tether cable/commutator system and animals were free to move in their cages. Recordings lasted from 2 to 24 hours, beginning at light onset (07:00am) with ambient temperature maintained at 22 degrees Celsius. Neck EMG, EKG, respiration, and EEG were filtered between 0.1 Hz and 4 kHz, digitized at 20 kHz, and continuously displayed and archived using a custom data system (Rector and George, 2001). Digital filtering, using infinite impulse response (IIRC) Butterworth filters, was used to view and analyze the channels at appropriate frequency ranges. The 20 kHz sample rate was necessary to resolve small changes in the high frequency components of the EEG and EMG across state.

To assess auditory evoked response characteristics across sleep-waking states, auditory click stimuli were produced with an 8 ohm speaker mounted approximately 30 cm above the animal's cage and connected to a pulse generator which created 5V, 0.2 ms square wave pulses (50 dB intensity) at 1 to 2 s random intervals throughout the recording. The clicks were sufficiently quiet so that the animal did not awaken during the stimulation, as determined by arousal coincidence with stimuli, but sufficiently loud so that a pronounced evoked response was apparent on the EEG, as recorded by each pair of parietal electrodes. After scoring the record for sleep (described in detail below), each auditory click event was assigned to a state, then time-triggered averages of the EEG displayed the average ERP for each state. Software for parsing the physiological record was initially written and tested using OCTAVE (www.octave.org), a freely-available mathematical and data analysis tool compatible with MATLAB (Mathworks, Inc, Natick, MA). For speed and efficiency, the code was converted to C under the Linux operating system using the 'Gnome Toolkit' (GTK, www.gtk.org) as the user interface.

Data Analysis

After the physiological signals were collected, we divided the data into successive 2 s epochs, and all channels for each epoch were converted into the frequency domain using FFT procedures. All records were visually scored in 10 s epochs into wake, quiet sleep, REM sleep, and indeterminate sleep stages by at least five trained people, scoring independently, for comparison with the cluster analysis method described below. The spectral data were then binned into total power for selected frequency ranges: All (0.1-4KHz), Delta (0.1-4 Hz), Theta

(4-8 Hz), Alpha (8-12 Hz), Sigma (12-16 Hz), Beta (16-25 Hz), and Gamma (25-45 Hz). In order to rapidly define states with 2 s epochs, each frequency range for each channel was plotted on an X-Y scattergram, initially with all epochs undefined. Time could also be used as a plot parameter for assessing trends over the course of the recording. We usually plotted EEG Delta power (Y axis) vs total power across all frequencies in the neck EMG (X axis), which provided the clearest clusters (Fig 1). We observed points in the scattergram that clustered tightly and were associated with different states as determined by visual scoring of the record. We manually drew limits around points which assigned particular states to the cluster. For scattergram scoring, 2 s epochs were chosen to maximize the number of points in each cluster and to capture brief states. Shorter epochs could be used, but did not change the cluster appearance, and limited our ability to compute low frequency components from the data.

The scattergram window was also smoothed using a Gaussian blurring convolution algorithm such that points in the scattergram became a blob. In those regions where a high density of points clustered, the blobs merged, forming larger dark regions. Regions with lower point densities appeared as lighter shades of gray. A gray-level histogram of this blurred image was then calculated, and topographical contour lines drawn at gray-level values representing 95%, 90%, 85% and 80% of all plotted values. In this way, clusters were statistically identified based on the density of points within each region.

Clusters associated with high EMG power and low EEG delta power were assigned to waking, low EMG power and high EEG delta power corresponded to quiet sleep, and very low EMG power with low EEG delta power represented REM sleep. This process was repeated for each state until all desired epochs were assigned. Usually, 80 to 85% of the epochs fell within well-defined clusters. For state verification, clicking a point in the scattergram recalled the physiological trace associated with that time and allowed review of the traces for that particular epoch. The traces were also color coded to match the assigned states. Similarly, clicking on the physiological traces highlighted the scattergram point associated with that time period. All epochs were visually scored to confirm assignment of each bin within the clusters. The window which displayed the traces was also used to manually adjust and/or fully score the record by pressing a key for each epoch that corresponded to a particular state. In this way, points that fell outside of well-defined clusters could be assigned. Scattered points between clusters usually represented transition states, and could be classified manually or left undefined. While the record was being scored in this fashion, a hypnogram that displayed sleep state across time was automatically generated.

Four types of analyses were performed to assess characteristics of auditory evoked responses across states and during individual epochs. First, to obtain an overview of the temporal characteristics of auditory evoked responses across state, the stimuli were sorted by sleep state, and the corresponding evoked responses averaged. For each averaged response, the amplitude and latency of the first peak (P1) and trough (N1) were measured and compared to values from ERPs in the active wake (AW) state across all animals, using a paired student t-test. Second, to assess the temporal characteristics of the auditory evoked response to individual stimuli, we employed a curve fitting routine to extract the evoked response from ongoing EEG. The curve fitting procedure was necessary for unbiased determination of each response, since individual evoked responses were highly variable. Third, the EEG during QS4 was filtered between 0 and 4 Hz to identify slow waves, then evoked responses were averaged based on their phase relationship to the slow waves. Finally, EEG slow waves were broken into 200 ms bins and evoked responses were averaged separately based on the total power of the high frequency components (40 to 2000 Hz).

Individual ERP characteristics

The curve fitting procedure followed three steps. First, the average evoked response was calculated for the entire recording. There were typically 4800 stimuli during each two hour recording period. The average response was then divided into two components consisting of the P1 response and the N1 response, and normalized into unit basis functions. The two basis functions were fit to each individual evoked response using a minimizing least-squares curve fit routine (adapted from CURVEFIT.PRO, IDL, ITT Visual Information Solutions, Boulder, CO) that optimized four parameters: P1 and N1 amplitude, P1 and N1 latency.

Once the fit parameters were obtained for individual responses, we plotted a histogram of the parameter values for each sleep state. To emphasize differences between sleep states, histograms for all states were subtracted from the active wake histogram. The differentiated P1N1 amplitude histograms showed a trimodal distribution of responses based on two inflection points. We classified the responses into three groups based on these points of inflection: low ($< 20\mu\text{V}$), medium (20 to $60\mu\text{V}$) and high ($> 60\mu\text{V}$) values, and average evoked responses were created for each state based on the three ranges. A similar set of averages was created for evoked responses that fell into three classifications based on the N1 time arriving early ($< 32\text{ms}$), intermediate (32 to 60ms) and late ($> 60\text{ms}$). Values were displayed as percentages because there were a different number of ERPs in each state, depending on the total duration.

ERP Phase Relationship to Delta Slow Waves

In order to assess the effect of slow waves on the evoked response amplitude, we selected three to five 10 min segments of quiet sleep with highest delta power from each animal (QS4) and digitally filtered the frontal EEG between 0-4 Hz. For each auditory stimulus, we identified the nearest trough in the filtered waveform and measured the time between the stimulus and the slow wave nadir (as described in Massimini et al., 2003). Stimuli were binned in 40ms intervals from the center of the slow wave nadir, up to 200 ms before and 200 ms after the nadir, and evoked response was averaged for each phase bin. This procedure created phase averaged ERPs with a large slow wave component superimposed on the ERP average. To remove the slow wave component from the ERP phase averages, the slow wave nadirs were used to create an average slow waveform across the epoch. The average slow waveform was offset in time and subtracted from each phase averaged evoked response to remove the contribution of the slow wave from the phase averaged ERPs. We then compared the P1N1 amplitude for each phase averaged ERP to the ERP average obtained at the slow wave nadir using a paired t-test.

ERP Relationship to High Frequency Components of Slow Waves

Since slow waves are characterized by intracellular fluctuations between depolarized and hyperpolarized states (Steriade et al., 2001), and since more spontaneous activity is present during the depolarized state, we examined evoked response amplitudes during EEG periods with high and low power in the high frequency components (Mukovski et al., 2007). For all animals, we selected quiet sleep periods with high slow wave activity (QS4), and used FFT procedures to calculate spectral power across each 1 Hz frequency bin from 0 to 2000 Hz in 200 ms epochs. We then calculated total power between 40 to 2000 Hz, and plotted a histogram of high frequency power verses the number of bins at each power. The resulting histogram exhibited a bimodal distribution since the high frequency power was low during the hyperpolarized (quiet or down) state, and high during the depolarized (active or up) state of slow waves. Each stimulus was then sorted into those that occurred during low and during high power conditions, and the respective evoked responses were averaged together. This procedure was repeated for all animals, and a paired t-test was used to calculated significant differences in P1N1 amplitude.

Results

Cluster analysis of the FFT scattergrams revealed at least eight regions using the gray-level contour lines corresponding to at least eight unique sleep states (Fig 1). Hypnograms from the cluster scoring method with 2s and 10s epochs corresponded closely to visual scoring (Fig 2 and Table 1), with 89% (st. dev. = 3%) average concurrence between cluster scoring and five hypnograms, visually scored from different manual scorers and 86% (st. dev. = 4%) concurrence between the five visually scored hypnograms.

Upon close inspection of representative traces from each state (active wake: AW, wake: W, quiet wake: QW, quiet sleep 1: QS1, quiet sleep 2: QS2, quiet sleep 3: QS3, quiet sleep 4: QS4 and rapid eye movement sleep: REM), the EEG and EMG showed characteristic frequency patterns (Fig 3), and also demonstrated the difficulty in visually identifying differences in the EEG pattern between the four QS states seen in the scattergram (Fig 1).

When the auditory evoked responses were separated into the 8 different states, we observed a progressive increase in average P1 amplitude from AW, reaching a maximum during QS2, then decreasing in amplitude during QS3, QS4 and REM respectively (Fig 4, upper panel). The average N1 amplitude remained elevated during quiet sleep, and average N1 latency increased throughout the deeper levels of sleep, becoming shorter again during REM (Fig 4, lower panel). Figure 5 shows the distribution profiles of P1N1 amplitudes and the latency to the N1 trough of each individual evoked response, sorted across sleep state.

The P1N1 amplitude and latency distributions exhibited a trimodal distribution as evidenced by two points of inflection in the difference histograms (Fig 5) and fell into three categories based on changes in components across state, classified into low (< 20 uV), medium (20 to 60 uV) and high (> 60 uV) amplitude values and early (< 32 ms), intermediate (32 to 60 ms) and late (> 60 ms) latency to the N1 trough. When the ERPs were divided into the three categories and averaged, responses showed three types of evoked responses with specific temporal profiles (Fig 6). The average number of individual ERPs from all animals in each category across state is displayed in Figure 7. The distribution of individual ERP components was consistent across state, and corresponded to the overall averages across state shown in Figure 4. When the P1N1 amplitude fit parameter was less than 20uV (~8% of total responses), the average evoked response was absent, with only a negative deflection and a delayed peak after 100ms. A similar profile was observed for responses where the N1 trough fit parameter was less than 32ms. When the P1N1 amplitude fit parameter was greater than 60uV, or when the N1 latency was longer than 60ms, a prominent P1N1 peak was evident. During waking and REM sleep, the individual evoked responses were more consistent and smaller in amplitude, with short latencies. In QS1 and QS2, approximately half of the responses exhibited larger amplitudes and longer latencies than those typically seen during waking. In QS4, there were more responses lower than 20uV, but those that appeared had larger amplitudes and longer latencies.

Epochs of quiet sleep showed large slow waves which appeared as rhythmic oscillations of 0 to 4 Hz (Fig 3). When the EEG during slow wave oscillations was filtered between 0-4 Hz, the ERPs generated during this time could be sorted based on their relative phase relationship to the slow waves. While an alternating current (AC) coupled EEG signal can never be a direct measure of the underlying neural membrane potential, Figure 8 shows that during slow wave oscillations (i.e. when the AC component of the EEG is modulated by the population dynamics of membrane potential fluctuations from large numbers of cells acting synchronously below 4 Hz), the underlying mechanisms generating slow waves may relate to the membrane potential of both cortical neurons and thalamo-cortical cells (Steriade et al., 2001), and the EEG amplitude during slow wave oscillations could correlate with the relative membrane potential

changes of the neurons as a group. When evoked responses were averaged based on their phase relationship to the nadir of the slow waves, we found a 30% decrease in the PIN1 amplitude for those responses that occurred during the peaks of the slow waves ($p < 0.1$) with the largest ERPs occurring during the nadir of the slow wave (Fig 8).

In some animals, we also placed a sub-dural cortical surface electrode (ECoG) to record surface field potentials. One trace from a surface cortical electrode is shown in Figure 9A, illustrating slow wave potentials. When the surface potential was negative relative to our occipital reference electrode, we observed relative quiescence in the signal fluctuation as compared to a high level of fast fluctuations when the surface potential was positive. The spectral power of slow waves of one animal from 1 to 2000 Hz is shown in Figure 9B. The average spectral power of the high frequency fluctuation (depolarized, active or up) periods divided by the average spectral power of the quiet (hyperpolarized or down) periods is shown in Figure 9C. The power ratio shows a significant increase in the spectral power above 40 Hz, as was found in an earlier study (Mukovski et al., 2007). For each epoch during quiet sleep with high slow wave activity (QS4), the average power from 40 to 2000 Hz was calculated and plotted in a histogram (Fig 9D). The resulting histogram exhibited a bimodal distribution such that epochs during the quiet periods of the slow waves had low total power in the high frequency range, and periods with high frequency fluctuations had high power in the high frequency range. When evoked responses were sorted and averaged based on their low or high power bins (Fig 9E), the PIN1 amplitude was significantly higher during the low power, quiet periods of the slow waves (Fig 9F, $p < 0.05$).

Discussion

Analysis of individual ERPs show that the cortex remains responsive to external stimuli during sleep, and can generate synaptic events at least 90% of the time regardless of state. Only in deep quiet sleep (QS3 and QS4) did we observe a small decrease in the number of medium to large ERPs. Our data support the hypothesis that evoked responses become larger during QS due to increased numbers of large individual responses. Since the evoked response size is dependent on slow wave phase (Fig 8 and Massimini et al., 2003), large evoked responses may occur more often during QS because cortical cells are hyperpolarized more often, when compared to waking (Steriade et al. 2001; Timofeev et al., 2001). Since delta waves appear with roughly a 50% duty cycle, and are probably generated by either synchronized thalamo-cortical or cortico-cortical cell oscillations between hyperpolarized and depolarized states (Steriade et al. 1993, 2001; Kisley and Gerstein 1999; Shu et al., 2003), the distribution of evoked response amplitudes during quiet sleep parallel the expected delta wave distributions (Fig 8 and Massimini et al., 2003). Detection of hyperpolarized and depolarized states from the EEG may also be determined by looking at the spectral composition of the EEG from 20 to 100 Hz (Mukovski et al., 2007). When cells within the cortical column are in their depolarized state, they exhibit a higher incidence of spontaneous activity. Our data suggests that frequencies between 200 and 2000 Hz could also be useful in detecting hyperpolarized and depolarized states (Fig 9).

The detailed mechanisms that underlie the larger ERP during quiet sleep require additional studies of intracellular membrane potential during wake and sleep. Nevertheless, several possibilities could explain the present results. For example, when cortical cells are activated from their hyperpolarized state, the synaptic event may elicit a depolarization which may reach the same final membrane potential as the depolarized state, as determined primarily by the sodium Nernst potential. Thus, the synaptic event may generate a larger change in membrane potential in quiet sleep than during waking, because the membrane potential traverses a larger change in voltage. Since synaptic responses between cortical neurons contribute strongly to the N1 component of the evoked response (Jellema et al., 2004), a lower membrane potential

could also contribute to a delay in the N1 response. A group of cortical cells in their hyperpolarized state may also exhibit an increased probability of synchronous firing, resulting in a larger ERP recorded at the cortical surface. Finally, thalamo-cortical cells are typically hyperpolarized during quiet sleep (Hirsch et al. 1983), and may generate a bursting response that could be reflected at the cortical level as larger amplitude synaptic response and, as a consequence, a larger amplitude evoked potential. Their depolarization may also take longer, explaining longer latency of cortical response (Rosanova and Timofeev, 2005).

K-complexes represent another waveform that could influence evoked response shape and amplitude. The presence of K-complexes in the EEG during slow wave sleep contributes to the delta band (1-4 Hz) (Amzica and Steriade, 1997), and can be evoked by external stimuli (recently reviewed in Bastien et al., 2002). Since K-complexes are much higher in amplitude, slower and distinct from ERPs, with a different waveform, they may indeed be generated by bursts from thalamo-cortical neurons. While the mechanisms that generate K-complexes may be similar to those involved in both slow wave generation and the state-dependent changes in the evoked response, K-complexes are slower and much longer lasting than the P1 and N1 components of the evoked response. In the present study, we did not analyze late components typically associated with K-complexes.

We found that the average ERP was larger during light sleep (QS1 and QS2) because a larger proportion of individual ERPs with high amplitude responses occurred during this state. Earlier reports of the evoked response size across sleep states have been inconsistent, with some investigators reporting smaller evoked responses during sleep (Shaw et al. 2006), and some reporting larger evoked responses during sleep (Weizman and Kreman 1965; Mendel and Goldstein 1971; Howe and Serman 1973; Pena et al. 1999; Rector et al. 2005). However, none of the earlier studies separated the evoked responses into as many different sub-states as the present study, and the earlier studies used longer epochs to score sleep. Indeed, if AW, W, and light quiet sleep (QS1 and QS2) were excluded, as in some studies (Shaw et al. 2006), and comparisons made between QW and deep quiet sleep (QS3 and QS4), then the evoked responses might appear to decrease during quiet sleep (Fig 4). Our study shows that the size of the evoked response differs across the four quiet sleep stages, with the greatest occurrence of large evoked responses (P1N1 amplitude >60uV and N1 trough >60ms) during QS2.

During deeper quiet sleep (QS4), evoked responses appeared lower, perhaps because either cortical or thalamic cells were hyperpolarized to the point where it was more difficult to initiate an evoked response (Livingstone and Hubel, 1981). Thus, the averages might be smaller because more trials (9% in QS4 vs 6% in QS2) generated no response (Fig 6 and 7). Our results show that most external stimuli generate cortical evoked responses, in spite of thalamic sensory gating (McCormick and Bal, 1994). While much of the ongoing or spontaneous activity may not get through the thalamic gate due to their hyperpolarized state, most stimuli we have attempted to date, including somatosensory, visual and auditory, appear as evoked responses in the cortex. Etiologically, this may allow the animal to respond to important cues during sleep while suppressing ongoing spontaneous activity or noise. Other evidence shows that the cortex itself can modulate thalamic bursts in response to novel stimuli, at least during waking (Fanselow et al., 2001), thus bursts are not exclusive to sleep states. To provide additional evidence for this hypothesis, further investigation is required, using intracellular or voltage sensitive dye recordings of membrane potentials during evoked responses across sleep states.

In this study, cluster analysis techniques provided a powerful tool for scoring sleep states, especially when using short, 2 s epochs. Direct comparison of the hypnograms from visual and scattergram scoring showed good correspondence in state determinations, and in a fraction of the time required with visual scoring. Cluster analysis revealed four different stages of quiet sleep and three types of waking in rodents which could not be readily observed with visual

scoring. Additionally, topographical boundary techniques identified clusters and provided an objective view of the data to identify states. When ERPs were separated into the eight different states, clear differences in the ERP temporal signatures appeared, further supporting the state divisions that appeared in the cluster analysis. While it is difficult to make direct comparisons between the four QS stages observed here and human quiet sleep, we observed a consistent progression from QS1 to QS4 in the hypnograms suggesting some similarities. Since rodents have fewer cortical neurons, delta rhythms might appear different between rats and humans and changes between the four quiet sleep stages might be more subtle. Analysis using additional parameters, such as spindles, could be used to further characterize state differences.

Since the ERP temporal signature distributions followed the sleep state with a high level of significance across all animals studied (Fig. 7), it is tempting to consider using the evoked response to assist scoring of sleep. Unfortunately, this strategy was not successful, since evoked responses of any shape can occur during all states. For example, if whole animal sleep were scored solely based on the size of the evoked response, the sleep state would be misclassified half of the time in quiet sleep, since the ERP is small approximately half of the time. Our earlier work (Rector et al. 2005) showed that the ERP might be used to determine whether a local brain region is in its sleep-like state, independently from whole animal sleep and may be regulated by homeostatic mechanisms (Feinberg et al. 1985). Thus, ERP amplitude fluctuations during waking and sleep could reflect localized state changes within cortical columns, as evidenced by a measure of use dependence (Rector et al., 2005). At least one review suggests that the the depolarized (up) state could reflect fragments of wakefulness, and is modulated by T-type calcium channels (Destexhe et al., 2007). Additional studies that drive specific cortical columns more or less than others are required to test the use-dependence of ERP amplitude.

In summary, we compared evoked response characteristics across rat sleep that may correspond to eight stages of human sleep. Each of the stages shows functional differences in the size of cortical auditory evoked responses that correspond to findings in humans (Weitzman and Kremen 1965; Mendel and Goldstein 1971) and may result from state related differences in the temporal distribution of hyperpolarized and depolarized states. A better understanding of the state dependent relationship between ERP signatures and cortical function could provide new insights into the function and regulation of sleep.

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List of abbreviations

ERP	Evoked response potential
AW	Active wake
W	Wake
QW	Quiet wake

QS	Quiet sleep
REM	Rapid eye movement (sleep)
EEG	Electroencephalogram
EMG	Electromyogram
EKG	Electrocardiogram
Delta	0.1 to 3Hz rhythm
IIRC	infinite impulse response filter
FFT	Fast fourier transform
P1	First peak of the evoked response
N1	First trough of the evoked response

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Figure 1.

The electrophysiological record for each animal was divided into 2 s epochs and FFT procedures were used to calculate the power spectrum of different frequency ranges. The panels represent a scattergram from a two hour period of one animal, neck EMG power across all frequencies against the frontal lobe EEG Delta power (0.1 to 4Hz) with 2 s epochs. The upper panel shows a scattergram image smoothed with a 50 point Gaussian blurring routine, and four contour lines drawn at iso-intensity levels encompassing 95, 90, 85 and 80% of the scattergram points respectively, defining distinct clusters of points. After smoothing, the original scattergram points were superimposed on the image to illustrate the original density of the points. At least eight states including: active wake (AW), wake (W), quiet wake (QW), quiet

sleep 1 to 4 (QS1, QS2, QS3, and QS4) and rapid eye movement (REM) sleep can be identified in the scattergram. Points that fall outside of the clusters usually represented transition states.

Figure 2.

Hypnograms of the data shown in Figure 1 show close correspondence between visual scoring of 10 s epochs (lower trace) compared to cluster scoring (middle trace) by drawing regions around the clusters of Figure 1. Cluster scoring of 2 s epochs (upper trace) shows a similar pattern in the hypnogram as the 10 s scoring method, but with greater sensitivity to brief state changes that are shorter than 10 s, and better discrimination between levels of quiet sleep.

Figure 3.

Representative frontal lobe EEG, neck EMG and EKG are plotted for each of the 8 states identified by cluster analysis using 2s epochs in Figure 1. These examples were taken from an epoch in the center of each cluster. Waking and REM states are clearly identifiable by the high frequency, low amplitude EEG with neck EMG nearly absent during REM. The four quiet sleep states all show higher amplitude EEG with lower frequency components and decreasing neck EMG levels from QS1 to QS4; however, it would be difficult to discriminate these four states by visual parsing of the record.

Figure 4.

Each auditory evoked response from data in Figure 1 was sorted by state as identified by cluster analysis, and averaged together to form an average evoked response for each state (upper panel). The same procedure was followed for all animals, and the corresponding average evoked responses were normalized in amplitude to the active wake (AW) condition. For the average response from each state, we then measured the P1 and N1 amplitudes and the P1 and N1 latencies for each animal and compared the values across state (lower panel, * = $p < 0.01$). The resulting bar graphs show that P1 amplitude increased from AW to QS2, but dropped back down to waking (W) levels during QS4 and dropped even further to AW levels during REM. P1 latency did not significantly differ across states. The N1 amplitude and latency increased from AW to QS4, dropping down again during REM.

Figure 5.

The P1N1 amplitude and latency to N1 for each individual evoked response were measured using a curve fitting routine and separated by sleep state. For the active wake (AW) state, we plot the distribution of amplitude and latency as a percentage of the total number of stimuli (% Total Stimuli). To illustrate differences in amplitude and latency distribution across state, we show the change the distribution from AW for each bin. As sleep progressed from AW to QS4, the fraction of individual evoked responses with an intermediate amplitude decreased, and the fraction of larger responses increased. The fraction of individual responses with intermediate N1 latency decreased and the fraction of longer latencies increased. The change in the distributions exhibited a trimodal pattern that can be seen best in the QS2 data. Vertical dotted lines represent the divisions of the distributions at 20 and 60 uV for the P1N1 amplitude components, and at 32 and 60 ms for the N1 latency. During QS4, we observed an increase in the fraction of low amplitude or non-existent responses.

Figure 6.

The ERP distributions shown in Figure 5 were divided into three categories for P1N1 amplitude: less than 20uV, between 20 and 60 uV, and greater than 60 uV. The responses that fell in each category were averaged together by state and plotted to show the characteristic shape of the responses in each category. The percentage of the total stimuli that fell into each category within each state is indicated next to the traces. Similarly, the N1 trough distributions were divided into: less than 32 ms, between 32 and 60 ms, and greater than 60 ms. The evoked responses that had low amplitudes and short N1 latencies have a characteristic temporal structure with an initial negativity and minimal P1 component. The evoked responses with large amplitude and late N1 components were significantly larger than the average waking response.

Figure 7.

The total percentage of evoked responses across state shown in Figures 5 and 6 were averaged over the three categories of P1N1 amplitude and N1 latency across all 10 animals. Significant differences from the AW state ($p < 0.01$) are marked with (*) and significant differences between QS4 and QS2 are marked with (&). Error bars are the standard error of the mean calculated from the average across all animals.

Figure 8.

During quiet sleep periods with high amplitude slow waves (QS4), we filtered the EEG between 0 and 4 Hz, then identified the time point for each nadir in the slow waves. By registering the nadir of each slow wave in time, we created an average slow wave trace (top trace). The gray region around the average slow wave represents the standard error of the mean for each sample point. For each stimulus, the evoked response was averaged based on its timing relative to the nearest nadir in 40 ms bins. Evoked responses that occurred during and up to 80 ms before or after the nadir were significantly larger ($p < 0.1$) than responses that occurred 120 to 160 ms before or after the nadir. Responses that occurred 200 ms before or after the nadir appeared to have high amplitudes, but the variability was also high and did not reach significance. Evoked response traces below the average slow wave represent average data from one animal with the average active wake (AW) and quiet sleep (QS2) ERP traces for comparison. The bar graphs on the bottom are the grand averages of P1N1 differences relative to the active wave state across all animals with standard error of the mean and significance (*) $p < 0.1$ identified.

Figure 9.

Subdural cortical surface electrodes (ECoG) were implanted in some animals which showed clear evidence of slow waves (A). During the negative phase of these slow waves, the signal is relatively quiet, with high frequency fluctuations during the positive phase. When the frequency spectrogram was calculated for the EEG during slow waves (QS4), a prominent peak was seen in the low frequency range, dropping off exponentially for higher frequencies (B). Spectrograms were calculated for each 200 ms time bin for at least 10 minutes of slow wave sleep, and divided into quiet (negative phase) and noisy (positive phase) periods. The ratio of the spectral power for noisy periods divided by quiet periods shows the largest increases in power occur between 40 and 2000 Hz (C). The total power between 40 and 2000 Hz for each 200 ms bin was calculated and plotted in histogram form (D) revealing a bimodal distribution representing quiet periods with low power in the high frequency range (gray) and high power in the high frequency range (black). Average evoked responses showed significantly higher amplitude ($p < 0.05$) during the low power (quiet, hyperpolarized or down, negative slow wave phase) period (E). The average differences in evoked response compared to the active wake average across all animals is shown in (F).

Sleep recordings were divided into 10 s epochs and scored for sleep state manually by five individuals, and by using the cluster cutting method. Inter-scoring agreement ranged from 81% to 95% and ranged from 86% to 93% for the cluster cutting method. Most of the differences occurred during transition states.

Table 1

	Score A					
Score B	85%	Score B				
Score C	84%		Score C			
Score D	89%	83%		Score D		
Score E	95%	88%	88%		86%	Score E
Cluster Score	88%	83%	81%	86%	93%	87%