An Oscillatory Hierarchy Controlling Neuronal Excitability and Stimulus Processing in the Auditory Cortex

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ABSTRACT.

Electroencephalographic (EEG) oscillations are hypothesized to reflect cyclical variations in the neuronal excitability, with particular frequency bands reflecting differing spatial scales of brain operation. However, despite decades of clinical and scientific investigation, there is no unifying theory of EEG organization and the role of ongoing activity in sensory processing remains controversial. The present study analyzed laminar profiles of synaptic activity (current source density, CSD) and multiunit activity (MUA), both spontaneous and stimulus-driven, in primary auditory cortex of awake macaque monkeys. Our results reveal that the EEG is hierarchically organized; delta (1-4 Hz) phase modulates theta (4-10 Hz) amplitude, and theta phase modulates gamma (30-50 Hz) amplitude. This Oscillatory Hierarchy controls baseline excitability and thus, stimulus-related responses in a neuronal ensemble. We propose that the hierarchical organization of ambient oscillatory activity allows auditory cortex to structure its temporal activity pattern so as to optimize the processing of rhythmic inputs.
INTRODUCTION.

The electroencephalogram (EEG) has been widely used for over 75 years as a measure of human brain function (see e.g. Berger, 1929). However, because of the dynamic complexity of the EEG, our understanding of its control and functional significance remains rudimentary. Recent studies have begun to link specific brain operations to specific components of the EEG, including “gamma” (Singer and Gray, 1995; Bertrand and Tallon-Baudry, 2000; Engel et al., 2001; Fries et al., 2001b), “theta” (Chrobak et al., 2000; Kahana et al., 2001; Buzsaki and Draguhn, 2004), and “alpha” (Worden et al., 2000; Makeig et al., 2004). The present study exploits and extends these findings.

Our study focused on three issues. The first issue is the intrinsic oscillatory “structure” of the EEG. There is an apparent relationship between theta oscillatory phase and gamma oscillatory amplitude in hippocampus (Bragin et al., 1995; Buzsaki et al., 2003; Csicsvari et al., 2003) and entorhinal cortex (Chrobak and Buzsaki, 1998; Cunningham et al., 2003). Human recordings also show that periodic high frequency oscillations are nested in lower frequencies and appear rhythmically in the spontaneous EEG (Freeman and Rogers, 2002; Vanhatalo et al., 2004). We predict a more extensive systematic dependence of higher frequency oscillatory amplitude on lower frequency phase than that outlined by these studies. The second issue is the relationship between the EEG and neuronal excitability. EEG oscillations are hypothesized to reflect cyclical variation in the excitability of neuronal ensembles (Bishop, 1933), but this idea has been explored mainly with regard to the low (<1 Hz) frequency oscillations (Steriade et al., 1993; Contreras et al., 1996; Sanchez-Vives and McCormick, 2000). The final issue is the effect of the ambient EEG on sensory processing. While ongoing activity apparently can influence sensory responses (Arieli et al., 1996; Polich, 1997; Fries et al., 2001a; Truccolo et al., 2002; Kruglikov and Schiff, 2003; Massimini et al.,
2003; Fiser et al., 2004), the extent of these effects and mechanisms by which they are controlled are poorly understood.

We analyzed both the spontaneous and stimulus-driven oscillatory activity present in cortical laminar profiles of field potentials and concomitant multiunit activity (MUA). These profiles were recorded with linear array multielectrodes during penetrations through primary auditory cortex (A1) in awake rhesus monkeys. Rather than analyzing the laminar field potential distribution itself, we analyzed its second spatial derivative, the current source density (CSD) profile, because this approach eliminates effects of volume conduction and allows more direct interpretation of field potential oscillations in terms of the underlying synaptic activity in the local neuronal ensemble (Nicholson, 1973; Schroeder et al., 1995). We introduce a novel hypothesis about the “hierarchical” organization of EEG oscillations, the “Oscillatory Hierarchy Hypothesis.” This hypothesis states that the amplitude of the oscillations at each characteristic frequency (gamma, theta, etc.) is modulated by the oscillatory phase of a local lower frequency oscillation. We present evidence that the phase of these spontaneous oscillations controls the excitability of the local cortical neuronal ensembles and thus, strongly influences stimulus processing. Because the oscillatory hierarchy can entrain to repetitive stimulation, ambient activity can adapt so as to optimize the processing of rhythmic components present in many natural acoustic stimuli.

METHODS

Surgery. 4 male macaques (Maccaca mulatta), weighing 5-7 kg were prepared for chronic awake electrophysiological recording. All procedures were approved in advance by the Animal Care and Use Committee of the Nathan Kline Institute. Prior to surgery, each animal was adapted to a custom fitted primate chair and to the recording chamber. Surgery
was performed under anesthesia (1-2% isoflurane), using standard aseptic surgical methods (Schroeder et al., 2001). The tissue overlying the calvarium was resected and appropriate portions of the cranium were removed. The neocortex and overlying dura were left intact. To allow electrode access to the brain, and to promote an orderly pattern of sampling across the surface of the auditory cortices, matrices of 18 gauge stainless steel guide tubes were placed over auditory cortex. These matrices were angled so that the electrode track would be perpendicular to the plane of auditory cortex, as determined by pre-implant MRI (Schroeder et al., 1998). They were placed within small, appropriately shaped craniotomies, to rest against the intact dura. The matrices, along with socketed Plexiglas bars (permitting painless head restraint), were secured to the skull with titanium orthopaedic screws and embedded in dental acrylic.

**Electrophysiological recording.** Recordings were made in an electrically shielded, sound-attenuated chamber with SONEX ProSPEC Composite™ sound absorbing foam. Laminar profiles of field potentials (EEG) and concomitant action potentials (multiunit activity or MUA) were recorded during 25 experiments in 4 awake rhesus monkeys (number of experiments in the individual monkeys: 5, 8, 6, 6), using a linear array multi-contact electrode (24 contacts, 100 μm intercontact spacing) positioned to sample from all the layers simultaneously (Schroeder et al., 2001)(Fig.1A). Signals were impedance matched with a pre-amplifier (10x gain, bandpass dc-10 kHz) situated on the electrode, and after further amplification (500x) the signal was split into field potential (0.1-500Hz) and MUA (300-5000Hz) range by analogue filtering. Field potentials were sampled at 2kHz/16bit precision, MUA was sampled at 20kHz/12bit precision. Additional zero phase shift digital filtering (300-5000Hz) and rectification was applied to the MUA data, and finally it was integrated down to 1 kHz (sampled at 2 kHz) to extract the continuous estimate of cell firing. One-
dimensional CSD profiles were calculated (Fig. 2A) from the spatially smoothed (Hamming window) local field potential profiles using a three-point formula for estimation of the second spatial derivative of voltage (Nicholson and Freeman, 1975; Ulbert et al., 2001). CSD analysis provides an index of the location, direction, and density of transmembrane current flow, the first-order neuronal response to synaptic input (Schroeder et al., 1998).

**Experimental design.** In each of the 25 experiments, the laminar CSD profile evoked by binaural Gaussian noise bursts was used to position the multielectrode array to straddle the auditory cortex from the pial surface to the white matter (Schroeder et al., 2001). Once the position was refined, it was left stable for the duration of recording. In this study, the subjects were conditioned to sit quietly, but were not required to attend or respond to the stimuli. During recording, they were monitored continuously using electroencephalographic recording (EEG) and infrared video, and were kept in an alert state by interacting with them. At the beginning of each experimental session, after refining the electrode position in the neocortex, we determined the best frequency of the area using “suprathreshold” method (Steinschneider et al., 1995; Schroeder et al., 2001)) consisting of 70dB SPL 100ms (4ms rise/fall time) Gaussian noise bursts and pure tones (frequencies: 0.5kHz, 1kHz, 2 kHz, 4 kHz, 8 kHz, 16 kHz and 20 kHz). Auditory stimuli were produced using Tucker Davis Technology’s System III coupled with ES-1 speakers. After determining the best frequency offline, we recorded 2 minutes of spontaneous activity followed by an approximately 2 minutes long stimulus train (n=150) consisting of the best frequency pure tone with an inter-stimulus interval of 767 ms. We repeated this if there was any sign of the monkey moving during the recording, and only analyzed artefact free recordings.

**Data analysis.** Data were analyzed offline using Matlab (Mathworks, Natick, MA). After selectively averaging the CSD and MUA responses to a series of seven randomly
presented pure tones and Gaussian noise, we functionally assigned each recording site to A1 versus belt auditory cortex (Steinschneider et al., 1995; Schroeder et al., 2001; Fu et al., 2004). In the present study only recordings obtained from area A1 were used. Our data set consisted of two parts: 1) 2 minute long spontaneous recordings and 2) event related responses evoked by a stimulus train (n=150, ISI=767 ms) of best frequency pure tones (also recorded continuously and epoched off-line from -2000 to 2000 ms to avoid edge effects of the wavelet transformation). For the analysis of spontaneous and event related oscillations, instantaneous power and phase were extracted by wavelet decomposition (Morlet wavelet) on 84 scales from 1 to 101.2 Hz (Fig. 2B). After applying the wavelet transformation we calculated the square root of the power values to estimate the amplitude of the local oscillation currents (Fig. 2B, 2C). To compute oscillatory amplitude or MUA amplitude as a function of oscillatory phase, we sorted the phase values obtained from the wavelet transformation for the entire 2-minute recording from $-\pi$ to $\pi$ radians (Fig. 2D lowest trace). Then we applied the permutation vector obtained from sorting the phases to the oscillatory amplitude values and the MUA for the same segment (Fig. 2D upper two traces). This means we rearranged the oscillatory amplitude and MUA time series using the data gained from sorting the phase values from $-\pi$ to $\pi$ radians. If for example the 5th data point is closest to $-\pi$ in the original phase series, in the rearranged phase series it will be the first, and after applying the permutation vector gained from rearranging the phase values, the 5th point of the oscillatory amplitude time series and concomitant MUA will also be the first in the rearranged series. After rearranging, the data were then down-sampled to 360 data points.

Pooled amplitude and frequency values were evaluated statistically by ANOVA. When significant changes were detected with ANOVA, multiple comparisons (Statistical Toolbox of Matlab) were used to determine which pairs of means were significantly different. Phase
values were analyzed by circular statistics methods. Pooled phases were compared using the Watson $U^2$ test for circular data. Significant deviation from uniform (random) phase distribution was tested with Rayleigh’s uniformity test.

RESULTS.

**Laminar profile of spontaneous oscillations in area A1 of the auditory cortex**

Spontaneous rhythmic activity was observed in all cortical layers, but four locations had higher amplitude oscillations than the surrounding ones (Fig. 1D and 1E). Two of these locations were in the supragranular layers (‘$S_1$’ and ‘$S_2$’), one in the granular layer (‘$G_1$’) and one in the infragranular layers (‘$I_1$’). For each penetration these sites were identified empirically by noting the peaks in the spontaneous oscillatory spectrum (e.g., Fig. 1D). Across penetrations, mean distance was 324 $\mu$m between the $S_1$- $S_2$ (STD=88), 488 $\mu$m between the $S_2$- $G_1$ (STD=112) and 364 $\mu$m (STD=86) between the $G_1$- $I_1$ electrodes. Subsequent analyses focused on these sites.

The spontaneous CSD in each site contained three spectral peaks, in the delta, theta and gamma frequency ranges; this is illustrated using the amplitude spectrum of the $S_2$ site (Fig. 3). As shown in figure 1E, amplitudes of theta and delta oscillations in the supragranular layers were significantly greater ($p<0.05$) than in granular and infragranular layers. There was no significant difference in gamma amplitude in the supragranular and granular layers, but it was significantly smaller ($p<0.05$) in the infragranular layers than in more superficial sites. The mean frequency of oscillation within each band did not differ significantly across layers.
Hierarchical Organization of Spontaneous Oscillations

In the spontaneous activity, theta and gamma frequency oscillation currents showed large periodic amplitude fluctuations, and these had systematic patterns: gamma oscillation current density fluctuated at theta frequency, while the theta oscillation current density fluctuated at delta frequency. Further, the highest amplitude theta oscillations occurred at a specific phase of the delta oscillation (the rising – positive going part of the oscillation on electrode $S_2$) and the same relationship held between the gamma oscillation amplitude and the theta oscillatory phase. We named the phase of the lower frequency oscillation where the largest amplitude higher frequency oscillations occur the “bursting phase” of the wave. This is illustrated using activity from the $S_2$ site (Fig. 4A, upper two traces). These effects occurred in all layers (the oscillatory amplitude difference between bursting and opposite phase was significant for all frequencies, Wilcoxon signed rank, $p<0.01$), but were greatest in supragranular sites, as the upper graph of Figure 4B shows. We noted a periodic 0.05-0.2 Hz fluctuation in the amplitude of the delta waves, but technical constraints (data were digitized after passing the analog signal through a high-pass filter with a corner frequency of 0.1 Hz) precluded quantitative analysis here.

We also examined the relationship between the phase of delta, theta and gamma frequency oscillation currents and MUA (Fig. 4A, lower three traces). There was a clear phase related modulation of the MUA in all the layers (the MUA difference between the phase with maximal MUA, the “firing phase,” and opposite phase was significant for all frequencies, Wilcoxon signed rank, $p<0.01$), with the modulation being greatest in the granular layer (Fig 4B, lower graph). In the case of delta and theta oscillations, the firing phase appeared to lag slightly behind the bursting phase, but there was no significant difference between the two angular means (Watson $U^2$, $p>0.05$).
**Entrainment of delta oscillation to stimulus presentation**

The event-related CSD at the peak of the initial transient cortical response had three clear amplitude maxima (Fig. 5A), similarly to the spontaneous spectrogram (Fig. 3). While there were no significant differences between spontaneous and stimulus driven conditions in the frequencies of theta and gamma oscillations, the mean frequency of delta oscillations was significantly different in the two conditions (ANOVA, p<0.05). Strikingly, the peak frequency of stimulus-related delta oscillation was a near perfect match to the stimulus rate; in 22 out of 25 experiments the peak frequency in the delta band during stimulation was 1.33 Hz (the rate auditory stimuli were delivered at). Delta oscillation was different from the other two types in two more aspects: while stimulus-related theta and gamma oscillations were significantly larger (p<0.05) than the spontaneous oscillatory amplitudes, the amplitude of delta oscillation was significantly smaller (p<0.05). Also while there was a clear pre- to post-stimulus amplitude increase in the theta and gamma range, the amplitude of the delta oscillation showed no stimulus-related change (Fig. 5B). While the amplitude and frequency of spontaneous and stimulus related delta oscillations were significantly different, there was no difference in their laminar distribution (Fig. 6). This argues against the general possibility that spontaneous and entrained delta oscillations are generated by different neural elements.

These results indicate that although there was no stimulus evoked delta oscillation, the frequency of the ongoing delta waves rapidly adapted or “entrained” to the stimulation frequency. The entrainment is also indicated by the analysis of the pre-stimulus delta phase. That is, at the first stimulus in a train, delta phase was random (Rayleigh’s uniformity test, p<0.05, n=25), but repetitive rhythmical stimulation resulted in a non-uniform pre-stimulus
delta phase distribution in the rest of the trials (Rayleigh’s uniformity test, p<0.05, n=149). This result was characteristic of all 25 experiments (Fig. 7).

**Effect of delta phase on stimulus processing**

Do the above described rhythmical oscillation current and multiunit activity amplitude fluctuations represent excitability changes that have an impact on stimulus processing? To address this question, we analyzed the effect of pre-stimulus delta phase on the stimulus-related CSD and MUA. The analysis and the results are summarized in Figure 8. To measure the influence of the pre-stimulus delta phase on the single trial event related responses we created two variables for each individual trial. These were the rectified CSD and the MUA amplitudes in the 10 ms (response onset) to 100 ms (offset of the auditory stimulus) post stimulus interval (Fig. 8A). We found that the amplitude of the evoked response is influenced by the phase of ongoing delta oscillation. This is illustrated in 2 ways for a single experiment in figure 8. Figure 8B shows the distribution of single trial response amplitudes (CSD, left and MUA, right) as a function of delta phase. Delta phase was systematically related to stimulus-evoked response amplitude in all the cortical layers, with the modulation being largest in the supragranular layers (Fig. 8D). Figure 8C shows the laminar profile of response (CSD with superimposed MUA) to a pure tone, averaged across trials with “ideal” pre-stimulus delta phase (left) and “worst” pre-stimulus delta phase (right).

The “ideal” pre-stimulus phase, which resulted in the largest event related activation (φ mean=1.98 rad, φ dev=0.88) was counter-phase to the “worst” phase, which resulted in the smallest event related CSD and MUA (φ mean=-1.54 rad, φ dev=1.09). Statistical comparison of the ideal phase to the spontaneous bursting phase across all experiments revealed no
significant differences (Watson $U^2$, $p>0.05$). This fits with the idea that the bursting phase of the spontaneous oscillations represents increased excitability.

Effects were quantified across experiments using a Modulation Index (amplitude in ideal phase trials/amplitude in worst phase trials, Fig. 8D). Laminar differences in delta phase dependence are noteworthy. The extreme extent of delta phase dependence in the superficial layers indicates that processing in these layers is determined largely by “context,” that is, the instantaneous cortical “state” (in terms of frequency, amplitude and phase of ongoing activity) in which inputs arrive.

DISCUSSION

An early hypothesis (Bishop, 1933) suggested that spontaneous EEG reflects rhythmic variation of cortical excitability. Although the relationship of the EEG to neuronal activity was relatively neglected over the intervening years, recent studies have re-kindled interest in this topic. Intracellular recordings in carnivores provided a striking demonstration of neuronal membrane potentials undergoing slow rhythmic shifts between depolarized and hyperpolarized states during slow wave sleep (Steriade et al., 1993; Sanchez-Vives and McCormick, 2000). Other recent findings have pointed to an underlying structure to the EEG spectrum. As mentioned above: 1) In humans, cats and rats, higher frequency oscillations display amplitude fluctuations with periodicity matching that of lower frequency oscillations (Steriade et al., 1996; Amzica and Steriade, 1998; Freeman and Rogers, 2002; Lakatos et al., 2004; Vanhatalo et al., 2004), 2) Moreover, dependence of gamma oscillation amplitude on theta oscillatory phase is well characterized in rodent hippocampus (Bragin et al., 1995; Buzsaki et al., 2003; Csicsvari et al., 2003) and entorhinal cortex (Chrobak and Buzsaki, 1998; Cunningham et al., 2003), and 3) there is gathering evidence that ongoing cortical
activity has an effect on sensory processing (Jansen and Brandt, 1991; Arieli et al., 1996; Polich, 1997; Kisley and Gerstein, 1999; Fries et al., 2001a; Kruglikov and Schiff, 2003; Massimini et al., 2003; Fiser et al., 2004).

The present study provides a way to organize these important findings. First, we show that there is a hierarchical structure to the EEG, with amplitude at each oscillatory frequency being modulated by the phase of a lower frequency oscillation. This structure appears to extend from slow waves up through the gamma frequencies, although technical constraints in the present study precluded quantitative assessment of the inter-relationship of delta and very slow oscillations. Earlier findings from intracellular recordings in-vitro suggest that Layer 5 pyramidal cells play a key role in organizing and promoting slow oscillations in cortical neurons (Sanchez-Vives and McCormick, 2000). The fact that in our recordings, delta and theta oscillations are by far largest in the supragranular layers suggests that the pyramidal neuron ensembles there are also important in controlled cortical processing. The relationship between these findings remains to be established.

A second key aspect of our findings is that like the slow oscillation, the higher frequency oscillations reflect concerted excitability variations in cortical ensembles. This is reflected in local neuronal firing (MUA) which is clearly related to the phase of delta, theta and gamma oscillations.

Finally, we confirm that ambient oscillatory activity has significant effects on stimulus processing (i.e., stimulus-related activity), in that, for each band of oscillation, there are both ideal and worst phases, during which stimulus responsiveness is enhanced or suppressed. The fact that spontaneous and event-related oscillations occur in the same frequency bands, are both phasic, and have similar laminar distributions implies that they use the same neural circuitry. However, the question of whether the oscillatory hierarchy present
in spontaneous activity is preserved in stimulus related activity remains an important question for future investigations.

Our findings have important implications for cortical processing of natural acoustic stimuli. While stimulus processing clearly is structured by the ambient “context” (Arieli et al., 1996), the onset of a sound can instantly re-set the phase of the ambient delta oscillation, which effectively phase-locks the entire hierarchical structure of oscillatory activity to the stimulus. Thus, effects of ambient activity on cortical processing should be more dramatic for more complex rhythmic inputs that are typical of a natural environment. For example, resetting of the ambient oscillatory hierarchy should be enormously useful in processing sounds that occur with a period of 1-4 Hz, and have relatively phase-locked, rhythmic components at 4-10 Hz (theta) and 30-50 Hz (gamma). It so happens that for primates, including humans, the temporal structure of numerous biologically relevant stimuli (Singh and Theunissen, 2003), especially vocalizations (Shannon et al., 1995; Wang et al., 1995), fit this pattern remarkably well.

Acknowledgments: We thank Dr. Gyorgy Buzsaki and his laboratory for helpful discussions of methods and theory, and Drs. Zsuzsa Pincze and Csaba Rajkai for helpful comments on an earlier version of the manuscript. We also thank Tammy McGinnis, Monica O’Connell and Aimee Mills for their invaluable assistance in collecting the data. Finally, we want to acknowledge extremely valuable comments from an anonymous referee, during the review process. Correspondence and requests for materials should be addressed to C.E. Schroeder, Ph.D. (e-mail: schrod@nki.rfmh.org).
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FIGURE LEGENDS

Figure 1 - Laminar amplitude profile of spontaneous oscillation currents in area AI. A) For each experiment, field potentials were recorded with a linear array multi-contact electrode positioned to sample from all cortical layers. Laminar boundaries were determined based on functional criteria (see methods). B) A characteristic 1.5 second long spontaneous intracortical field potential recording. We applied one dimensional current source density (CSD) analysis to the field potentials obtained with the linear array multielectrode to estimate the locally generated intracortical synaptic currents. C) Color coded current source density (CSD) map of the spontaneous activity segment shown in panel B; CSD was derived from the field potential profile using a second derivative approximation (see methods). Current sinks (red) represent net inward transmembrane current flow in the local neuronal ensemble; sources (blue) represent outward currents. D) Laminar amplitude profiles of the spontaneous oscillation currents (delta, theta and gamma) gained from wavelet analysis of the spontaneous CSD segment (C) show two maxima in the supragranular layers (‘S1’ and ‘S2’), one in the granular (‘G1’) and one in the infragranular layers (‘I1’). These 4 peaks were reliably identifiable in all recordings. The sites were not in adjacent electrode channels (see results). E) Graphs show pooled data (all 25 experiments in 4 subjects) for the amplitude of spontaneous delta, theta and gamma band oscillations obtained from the two supragranular (S1, S2), granular (G1) and infragranular electrodes (I1). Error bars represent the standard error. Amplitudes of theta and delta oscillations in the supragranular layers were significantly greater than in granular and infragranular layers. Gamma amplitude was significantly smaller in the infragranular than in the supragranular and granular layers (see results).
Figure 2 – Estimating phase related power and multiunit activity (MUA). A) 3 second long spontaneous intracortical field potential (blue traces on the left) and concomitant MUA (red traces on the right) recording. To be able to analyze the locally generated intracortical oscillations, one-dimensional CSD profiles (color coded CSD map and overlaid white traces) were calculated from the spatially smoothed local field potential profiles. Channels with the largest spontaneous oscillations are marked S₁-S₂ (supragranular), G₁ (granular) and I₁ (infragranular). B) Spontaneous supragranular (S₂) CSD signal. We applied wavelet transformation to the CSD data to analyze the amplitude and phase of the spontaneous oscillations. The color coded time-frequency map below the CSD signal shows the amplitude of the spontaneous oscillations during the 3 seconds analyzed in this example. After time-averaging the wavelet amplitude results we got the spectrogram (blue trace to the right of the time-frequency map), and we were able to detect three obvious peaks at 1.4, 7.8 and 32 Hz (see also Fig. 3A). C) Upper blue trace shows the amplitude changes of the spontaneous 7.8 Hz (theta) oscillation in the analyzed segment, while lower orange trace shows the phase of the 1.4 Hz (delta) oscillation. D) To determine whether and how the phase of the 1.4 Hz oscillation is related to the amplitude changes of the 7.8 Hz oscillation, we sorted the phase values (lowest orange trace). Then we applied the permutation vector obtained from sorting the phase (of the 1.4 Hz oscillation in this example) to the oscillatory amplitude values (of the 7.8 Hz oscillation in this example) and the MUA that was recorded concomitant. Blue trace in the middle and red trace on top show the rearranged oscillatory amplitude and MUA values.

Figure 3 – Frequency and spectral amplitude of spontaneous oscillations. A) Grey trace shows a characteristic wavelet amplitude spectrum of the spontaneous oscillations recorded
from the lower supragranular electrode ($S_2$) in the same session shown in figure 1. Overlaid box and whisker plots show pooled data (all 25 experiments in 4 subjects) for the amplitude and frequency of the maxima of spontaneous oscillation currents in the delta, theta and gamma bands on electrode $S_2$ (box has lines at lower quartile, median, and upper quartile values, while whiskers show the extent of the data). Table shows pooled data ($n=25$) for all four laminar sites ($S_1$, $S_2$, $G_1$ and $I_1$). The mean frequency of spontaneous oscillations within each band did not differ significantly (for laminar amplitude differences see figure 1D).

**Figure 4 – Oscillatory phase, oscillatory amplitude and MUA relationships of the spontaneous oscillation currents.**

[A] Upper two traces show delta phase-theta amplitude and theta phase-gamma amplitude relationship in an individual experiment. Three cycles are shown for illustration purposes. Oscillatory amplitude is clearly modulated by lower frequency oscillatory phase. Bursting phase of the lower frequency oscillation is the phase with the maximal higher frequency oscillation amplitude. For example in the case of delta oscillation it is the phase where the largest amplitude theta oscillations occur. Overlaid box and whisker plot show pooled data for the bursting phase from all 25 experiments in 4 subjects (box has lines at lower quartile, median, and upper quartile values, while whiskers show the extent of the data). Lower three traces show modulation of concomitant MUA by the phase of the different frequency spontaneous oscillations in an individual experiment (same as above). Firing phase is the phase of the spontaneous oscillation currents during which cells are most likely to generate action potentials (largest MUA). Overlaid box and whisker plots show pooled data for the firing phase ($n=25$). All the data shown in the figure were recorded on the lower supragranular ($S_2$) electrode, but table shows pooled data ($n=25$) for the bursting and firing phases for all sites ($S_1$, $S_2$, $G_1$ and $I_1$).

[B] Phase dependent
oscillation current and MUA modulation in the supragranular (s1, s2), granular (g) and infragranular (i) layers. Upper box and whisker plots show pooled delta (to the left) oscillatory phase dependent theta amplitude modulation ratio, and pooled theta (to the right) oscillatory phase dependent gamma amplitude modulation ratio. Lower plots show pooled delta (left), theta (middle) and gamma (right) oscillatory phase dependent MUA modulation ratio. The ratios were calculated as: [mean amplitude of phase modulated oscillation currents/MUA in the pi/3 radian phase interval centred on the bursting/firing phase] / [mean amplitude of phase modulated oscillation currents/MUA in the pi/3 radian phase interval opposite to the bursting/firing phase]. While the phase related oscillation current modulation is largest in the supragranular layers, phase related MUA modulation is largest in the granular layer.

**Figure 5 - Frequency and spectral amplitude of event-related oscillations.** A) Grey trace shows the wavelet amplitude spectrum of stimulus-related CSD at the lower supragranular site (S2) 30 ms post-stimulus in the same experiment for which the spontaneous spectrum was shown in figure 3. Box and whisker plots show pooled data (all 25 experiments in 4 subjects) for the amplitude maxima and frequency of event-related oscillation currents in the delta, theta and gamma band. Outliers are marked by a red cross. B) Pooled (n=25) single trial wavelet amplitude data show the time-course of event-related oscillation amplitudes. Note that while theta and gamma oscillation currents show stimulus related increase, there is no change in the amplitude of the ongoing delta oscillation. The apparent pre-stimulus oscillatory amplitude increase in theta and gamma bands is a side-effect of the temporal smearing caused by the wavelet transformation.
Figure 6 - Laminar amplitude profiles of spontaneous and event related delta oscillation currents. The graphs on the left show laminar amplitude profiles of spontaneous and event related delta oscillation currents recorded in one experimental session (same as fig. 1). Both show two maxima in the supragranular layers, one in the granular and one in the infragranular layers. On the right bars show pooled data (all 25 experiments in 4 subjects) for the amplitude of spontaneous and event related delta oscillations obtained from the two supragranular (S₁, S₂), granular (G₁) and infragranular electrodes (I₁). Error bars represent the standard error of the mean.

Figure 7 – Entrainment of ongoing delta oscillation during rhythmic acoustic stimulation. Stacked bars on the left show the pre-stimulus phase of delta oscillation for the first trial of each trial block analyzed (25 trial blocks in 4 subjects). Different shades of grey represent the different trial blocks. Phase is measured at the time of stimulus presentation (0 ms) on supragranular electrode S₂. The distribution is random meaning that the phase of the ongoing delta oscillation is random at the time of the presentation of the first stimulus.

Stacked bars on the right show pooled data for the pre-stimulus delta phase in all succeeding trials (149 in each trial block) recorded in all 25 experiments. As in the previous graph, different shades of grey represent the different experimental sessions. The number of trials is normalized to the total number of trials (n=149) in each experimental session (n=25). It is obvious from the graph that the pre-stimulus delta phase in these trials is not random. This means that the ongoing delta oscillation is influenced (entrained) by the rhythmic acoustic stimulation.
**Figure 8 - Delta phase dependence of the event-related response.**

A) Characteristic laminar event related CSD (on the left) and MUA (on the right) profile. Channels with the largest event related oscillations are marked S₁-S₂ (supragranular), G₁ (granular) and I₁ (infragranular). To estimate the effect of pre-stimulus delta phase on the event related response we measured the rectified event-related CSD and MUA amplitudes for the 10-100 ms time interval (colored areas).

B) Event-related CSD and MUA amplitudes of trials with different delta phase in the same experiment. The event related response (CSD and MUA) is clearly dependent on the pre-stimulus delta phase in all cortical layers (sites S₁, S₂, G₁ and I₁). There is an ‘ideal phase’ which results in maximal event-related CSD and MUA (red stars) and there is a ‘worst phase’, which means that if pre-stimulus delta is in this phase, the event-related response will be much smaller (blue stars).

C) Event-related CSD profiles evoked by the best frequency tones; left - average of trials with delta phase corresponding to the largest post-stimulus activation (‘ideal phase’, red stars in panel B), right - average of trials with delta phase corresponding to the smallest post-stimulus activation (‘worst phase’, blue stars in panel B). Overlaid traces show MUA for supragranular (S₂), granular (G₁) and infragranular (I₁) electrodes.

D) Pooled data (all 25 experiments in 4 subjects) showing the modulation index (ratio of the ideal phase response over the worst phase response) for all cortical layers.
Figure 3

A) Spectral amplitude of spontaneous CSD

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<th>Electrode</th>
<th>amplitude (mV/mm²)</th>
<th>frequency (Hz)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>S₁</td>
<td>S₂</td>
</tr>
<tr>
<td>delta</td>
<td>mean</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>sd</td>
<td>0.19</td>
</tr>
<tr>
<td>theta</td>
<td>mean</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>sd</td>
<td>0.16</td>
</tr>
<tr>
<td>gamma</td>
<td>mean</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>sd</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Figure 4

A) Bursting phase of spontaneous oscillation currents

Firing phase of spontaneous oscillation currents

<table>
<thead>
<tr>
<th>electrode</th>
<th>bursting phase (rad)</th>
<th>firing phase (rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>delta</td>
<td>S₁, S₂, S₃, G, I, L₁</td>
<td>S₁, S₂, G, I</td>
</tr>
<tr>
<td>θ mean</td>
<td>-1.36, 1.62, 2.04, -0.37</td>
<td>-1.69, 2.22</td>
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<tr>
<td>θ dev</td>
<td>0.66, 0.04, 1.01, 1.03</td>
<td>0.81, 0.04</td>
</tr>
<tr>
<td>theta</td>
<td>&lt;θ mean&gt;</td>
<td>&lt;θ dev&gt;</td>
</tr>
<tr>
<td>gamma</td>
<td>-0.27</td>
<td>2.81</td>
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</tbody>
</table>

B) Phase dependent oscillation current modulation

Phase dependent MUA modulation

<table>
<thead>
<tr>
<th>electrode</th>
<th>theta dependent theta</th>
<th>theta dependent gamma</th>
<th>delta dependent MUA</th>
<th>gamma dependent MUA</th>
</tr>
</thead>
<tbody>
<tr>
<td>s₁ s₂ g i</td>
<td>electrode</td>
<td>electrode</td>
<td>electrode</td>
<td>electrode</td>
</tr>
</tbody>
</table>
Figure 8

A) Event related CSD

B) CSD amplitude

C) Ideal phase response

D) Modulation index by layer (CSD)

Event related MUA

B) MUA

C) Worst phase response

D) Modulation index by layer (MUA)