Analysis of stimulus-related activity in rat auditory cortex using complex spectral coefficients

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Krause BM, Banks MI. Analysis of stimulus-related activity in rat auditory cortex using complex spectral coefficients. J Neurophysiol 110: 621–639, 2013. First published May 8, 2013; doi:10.1152/jn.00187.2013.—The neural mechanisms of sensory responses recorded from the scalp or cortical surface remain controversial. Evoked vs. induced response components (i.e., changes in mean vs. variance) are associated with bottom-up vs. top-down processing, but trial-by-trial response variability can confound this interpretation. Phase reset of ongoing oscillations has also been postulated to contribute to sensory responses. In this article, we present evidence that responses under passive listening conditions are dominated by variable evoked response components. We measured the mean, variance, and phase of complex time-frequency coefficients of epidurally recorded responses to acoustic stimuli in rats. During the stimulus, changes in mean, variance, and phase tended to co-occur. After the stimulus, there was a small, low-frequency offset response in the mean and modest, prolonged desynchronization in the alpha band. Simulations showed that trial-by-trial variability in the mean can account for most of the variance and phase changes observed during the stimulus. This variability was state dependent, with smallest amount of information is discarded when only the evoked response is considered, because cortical responses are quite variable and this trial-by-trial variability may contain information about the circuit components responding to the stimulus and about their recent activity. Some of the latter information is captured in the “induced” response, which reflects poorly phase-locked additive components and rearrangements of ongoing activity that contribute to the variance but not the mean of the response (Klimesch et al. 1998; Tallon-Baudry et al. 1996).

The distinction between evoked and induced responses is of interest because of the postulated mechanisms generating these components. Evoked activity is thought to arise from feedforward propagation of sensory information through the cortical network and is typically associated with the response of a static network to input from the periphery. Under this model, the evoked response results from a linear combination of an invariant additive component with variable background activity. By contrast, the induced response is thought to arise from stimulus-related modulation of network parameters (e.g., connection strength, membrane properties) and/or activation of cortico-cortical or cortico-thalamo-cortical feedback loops (David et al. 2006; Pfurtscheller and Lopes da Silva 1999). By far the most common method for extracting the induced component is to obtain the residual after subtracting the averaged evoked component from the single-trial responses and then estimate its power (Kalcher and Pfurtscheller 1995; Klimesch et al. 1998). To the extent that this theoretical dichotomy holds, it is useful for assigning neural mechanisms to different response components. In reality, however, evoked and induced responses can become entangled in several ways, complicating their interpretation in terms of bottom-up vs. top-down and static vs. dynamic network properties. Nonlinear interactions between stimulus and ongoing activity can result in nonstationary background activity, and the evoked response itself can vary from trial to trial. Thus the residual is a combination of both components due to processes commonly ascribed to induced components, e.g., reorganization of ongoing activity and indirect triggering of oscillatory responses, as well as contributions due to trial-by-trial variability in amplitude and timing of the evoked response (David et al. 2006; Hu et al. 2009; Truccolo et al. 2002). Top-down processes that are reliably elicited by sensory stimuli will also appear in the evoked response (Picton 1992).

A third contributor to SRSPs is a phase reset of ongoing oscillatory activity. Phase synchrony has been proposed as a useful mechanism for facilitating integration between local neural assemblies (Varela et al. 2001). A phase reset produces an average time-domain response in the absence of an additive component. However, commonly used measures of phase reset such as intertrial coherence (ITC) (Lakatos et al. 2007; Makeig et al. 2002; Tallon-Baudry et al. 1996) are particularly susceptible to confounds due to additive components (Krieg et al. 2011; Martinez-Montes et al. 2008; Yeung et al. 2004), and other methods are susceptible to this confound, as well (e.g.,
Jansen et al. 2003; for a more complete discussion, see Krieg et al. 2011). Thus, as for evoked vs. induced components, phase reset and additive components can become entangled, complicating their interpretation, and the contribution of phase reset to auditory SRSPs remains controversial (Hanslmayr et al. 2007; Jervis et al. 1983; Krieg et al. 2011; Makeig et al. 2002; Makinen et al. 2005; Sauseng et al. 2007; Sayers et al. 1974). In this article, we present and discuss methods to separate these components that would be useful for simplifying their interpretation.

In particular, we show that trial-by-trial variability in an additive component can be manifested as changes in variance and phase, and thus open to misinterpretation. The primary means by which we show this is via simulations based on our recorded data. The purpose of analyzing simulated responses was twofold: first, these analyses provide a proof of principle that apparent induced and phase reset responses can be produced solely by trial-by-trial variability in the amplitude or latency of evoked components; second, a crucial benefit of having a generative or forward model of trial-by-trial variability is that we can address key questions about the relationship between the variability of evoked responses and background activity. We estimate the amplitude and latency variability of evoked responses by fitting the simulation model to empirical data features and then show that this variability is state dependent and correlates with electrophysiological measures of cortical arousal. This would not be possible without a generative model with which we could estimate the amplitude and latency variations.

Ongoing activity and SRSPs are broadband, and distinct frequency components have been associated with specific behavioral states, cortical loci, and circuit mechanisms (Buzsáki 2006). Thus substantial information about underlying cortical processes may be gained via spectral decomposition, most typically via time-frequency analysis (Jordan et al. 1997), which yields spectrograms that portray the evolution of frequency-specific activity over time relative to stimulus onset. The utility of analyzing complex spectral coefficients for disentangling evoked, induced, and phase reset response components has been highlighted recently (Krieg et al. 2011; Martínez-Montes et al. 2008). In this analysis, the set of coefficients at each time-frequency point recorded over many trials and plotted in the complex plane was shown to exhibit specific time-frequency activity that is indistinguishable and were pooled for analysis. The surgical procedures were as follows. Anesthesia was induced using isoflurane. After induction, animals were medicated with midazolam (3 mg/kg ip, to reduce the anesthetic requirement during surgery), the nonsteroidal anti-inflammatory agent ketoprofen (5 mg/kg sc, for postoperative swelling and pain), the opiate analgesic buprenorphine (0.05 mg/kg sc, for postoperative pain), and the local anesthetic bupivacaine (1 mg/kg sc, applied to the injection site and into the left or right temporalis muscle, depending on the implant site, for perioperative pain). Animals were then placed in a stereotaxic head holder (model 1900; David Kopf Instruments, Tujunga, CA). Anesthesia was maintained with isoflurane at 1.2–1.6% mixed with 50% O2 and 50% room air administered through a small rodent anesthesia mask, with the exact dosage adjusted to maintain an adequate surgical level as determined by immobility and lack of response to toe pinch. Animals were hydrated via subcutaneous injections of ~2 ml of 0.9% saline, and ophthalmic ointment was applied to prevent dehydration of the cornea. The animal’s body temperature was monitored using a rectal probe thermometer and was maintained at ~37°C throughout the surgery by wrapping the animal in a warm water blanket.

The fur over the dorsal skull was removed with a clipper and the skin disinfected with iodine solution and alcohol. An incision was made in the scalp overlying the dorsal surface of the skull along the midline, the skin was retracted to expose the skull, and subcutaneous tissue and peristium were retracted laterally. Membranous tissue was removed from the skull using a scraper. Self-tapping stainless steel bone screws (1.17-mm diameter; Fine Science Tools, Foster City, CA) were placed in the skull in the ipsilateral frontal, contralateral parietal, and ipsilateral occipital bones to assist in anchoring the electrode and interface board. A silver wire was attached to the screw in the parietal bone and served as a reference electrode. The ipsilateral temporalis muscle was dissected away from the skull and retracted laterally and ventrally using a three-pronged retractor to expose the skull ridge. Care was taken to minimize damage to the temporalis muscle to facilitate recovery after the surgery. A hole 4 mm in diameter was made in the temporal bone centered over the auditory cortex using a trephine (no. 18004-50; Fine Science Tools) while leaving the dura intact. Published stereotaxic coordinates of auditory cortex in the rat were used to determine the center of the craniotomy (Doron et al. 2002). The multielectrode recording array was lowered onto the dura and covered with dental acrylic, and the electrode interface board was cemented onto the top of the skull using dental acrylic to form a head cap. Buprenorphine (0.05 mg/kg sc every 12 h) and ketoprofen (5 mg/kg sc every 24 h) were administered for 3 days postoperatively for pain.

All experimental protocols conformed to American Physiological Society and National Institutes of Health guidelines and were approved by the University of Wisconsin Research Animal Resources Committee.

Electrode Preparation

Epidural electrode arrays were used for all electrophysiological recordings. The arrays consisted of 16 formvar-insulated stainless steel microwires (100-μm diameter; stainless steel 304; California Fine Wire, Grover Beach, CA) separated by 660 μm and arranged in 3 rows separated by 1,000 μm and embedded in a 4.0-mm-diameter, 2-mm-thick epoxy disc. Wires were cut flush with the surface of the epoxy disc, and the disc was ground with a curved burr bit to form a concavity that complemented the surface of the brain. The microwires were attached to an electrode interface board (EIB-16; Neuralynx, Bozeman, MT), which provided contact with a small profile connector (0.025 dual-row 18-pin connector; Omnetics Connector, Minneapolis, MN) for the amplifier head stage. The impedance of the electrodes at 1 kHz was typically 50–200 kΩ.

Electrode Implantation

Sixteen female rats 8–12 wk old were surgically implanted with electrode arrays over the left (n = 10) or right (n = 6) auditory cortex. Results from recordings from the right vs. left hemispheres were indistinguishable and were pooled for analysis. The surgical procedures were as follows. Anesthesia was induced using isoflurane. After induction, animals were medicated with midazolam (3 mg/kg ip, to reduce the anesthetic requirement during surgery), the nonsteroidal anti-inflammatory agent ketoprofen (5 mg/kg sc, for postoperative swelling and pain), the opiate analgesic buprenorphine (0.05 mg/kg sc, for postoperative pain), and the local anesthetic bupivacaine (1 mg/kg sc, applied to the injection site and into the left or right temporalis muscle, depending on the implant site, for perioperative pain). Animals were then placed in a stereotaxic head holder (model 1900; David Kopf Instruments, Tujunga, CA). Anesthesia was maintained with isoflurane at 1.2–1.6% mixed with 50% O2 and 50% room air administered through a small rodent anesthesia mask, with the exact dosage adjusted to maintain an adequate surgical level as determined by immobility and lack of response to toe pinch. Animals were hydrated via subcutaneous injections of ~2 ml of 0.9% saline, and ophthalmic ointment was applied to prevent dehydration of the cornea. The animal’s body temperature was monitored using a rectal probe thermometer and was maintained at ~37°C throughout the surgery by wrapping the animal in a warm water blanket.

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attenuation chamber (Industrial Acoustics, Bronx, NY). A small speaker (TDT-ES1; Tucker Davis Technologies, Alachua, FL) was mounted on the top (“ceiling”) of the enclosure, oriented toward the animal. The speaker was calibrated with a microphone (no. 4016; ACO Pacific, Belmont, CA) placed ~4 cm from the speaker, and stimuli were presented at ~75 dB SPL, assuming the animal’s head was this distance from the speaker. Speaker output varied by ±3 dB SPL over the range 10–20 kHz. A 16-channel preamplifier on a flexible tether (HS16; Neuralynx, Bozeman, MT) entered the enclosure and was attached to the animal’s head. The animal was able to move freely in the enclosure. Since the animal was unrestrained, actual stimulus levels on each trial varied slightly.

Free-field stimuli were applied using software (RPVDX; Tucker-Davis Technologies) triggered by the electrophysiological recording software (pClamp v8.2; Molecular Devices, Sunnyvale, CA). Stimuli consisted of 250-ms frequency-modulated (FM) sweeps (10–20 kHz) presented every 8 s for a period of 1 h. We note that this intertrial interval is long relative to most studies in which shorter, nonrandom intertrial intervals result in more predictable stimulus times and possibly minimize endogenous sources of response variability. We observed considerable variability of responses during the initial portion of the recording session as the animals became acclimated to the environment, and the data presented in this report represent the final 100 trials from each recording session. Sporadic remote video observations indicated that the animals typically were awake but immobile during this time period, but we did not verify the animal’s behavioral state on a trial-by-trial basis. Data were pooled across multiple recording sessions (n = 2–7 recording sessions for each animal, 1 session per day). Responses were bandpass filtered at 0.1–3,000 Hz, amplified 2,500× (Lynx 8; Neuralynx, Tucson, AZ), and digitized at 6.25 kHz (Digidata 1322A; Molecular Devices). Each trial consisted of a single stimulus presentation presented at time $t = 0$. For each trial, data were collected for 3,200 ms, beginning 658 ms before the stimulus.

Analysis of Electrophysiological Data

Recorded auditory responses were averaged, and the channel with the shortest latency and largest amplitude response (i.e., largest root-mean-square power computed during the time window 0 < $t$ < 300 ms) was assumed to be over primary auditory cortex and was chosen for analysis. We will refer to the single trial responses as $y_{i,k}(t)$, where $i = 1 \ldots L$, $k = 1 \ldots N_{\text{samples}}$, where $L$ is the number of trials and $N_{\text{samples}}$ is the number of sampled data points in a trial. Two criteria were used to reject trials contaminated by electrical or mechanical artifacts. The first criterion eliminated trials during which the head stage amplifier saturated following mechanical impact, e.g., with the walls of the recording chamber: $\mu_i > \mu_{\text{crit}}$, where $\mu_i = \frac{1}{N_{\text{samples}} \sum_{k=1}^{N_{\text{samples}}} (y_{i,k} - \bar{y}_i)^2}$ and $\mu_{\text{crit}}$ was set to 1 mV. The second criterion eliminated trials that had large spurious electrical and mechanical artifacts: $\sigma_i > \sigma_{\text{crit}}$, where $\sigma_i = \sqrt{\frac{1}{N_{\text{samples}} \sum_{k=1}^{N_{\text{samples}}} (y_{i,k} - \bar{y}_i)^2}}$, computed over the portion of the trial excluding the window 0 < $t$ < 1,000 ms (so as not to exclude trials due to unusually large responses), $N_{\text{crit}}$ is the number of sample points in the computation, and $\sigma_{\text{crit}}$ was set to 0.25 mV. In all experiments, <5% of the trials were rejected under these criteria.

Time-Frequency Analysis

We used time-frequency analysis to gain a detailed view of how specific frequency components contributed to the stimulus-related response and how the stimulus-related changes in these components related to ongoing activity. Time-frequency analysis of neural activity recorded over auditory cortex was performed using short-term Fourier transforms (STFT) for 5 frequencies from 3 to 16 Hz and continuous complex Morlet wavelet transforms (5 cycles) for frequencies from 16 to 300 Hz. STFTs were implemented by first downsampling the data by a factor of 4 (sample interval = 0.64 ms) and then tapering the data with a Hamming window and computing sliding Fourier transforms with a window width of 512 points and window overlap of 496 points. This choice of window width was a compromise between time and frequency resolution, and determined the minimum analysis frequency of 3 Hz. For the analysis of Fig. 11 in which we explored the state dependence of variability in the additive component of the response, we also calculated the coefficients at ~1.5 Hz by doubling the size of this window to 1,024 points. Prestimulus delta power for this analysis was then calculated by averaging the power at frequencies $f = 1.5$ Hz and $f = 3$ Hz. Wavelet transforms were implemented as described by Jordan et al. (1997), with 35 frequencies spaced exponentially to ensure 90% overlap in the pass band of adjacent Morlet filters. Transforms were computed for each trial for the initial 16,384 data samples (2,621 ms). The frequency boundary (16 Hz) at which we transitioned from STFT to wavelet analysis was chosen on the basis of the time resolution of the method, which was 164 ms for the STFT analysis and <164 ms (and improving with increasing frequency) for the wavelet analysis.

Statistical analysis of wavelet and STFT data was performed using a recently described method that performs tests on the complex coefficients computed on each trial for each frequency component (Martinez-Montes et al. 2008). Briefly, the time-frequency analysis yields a complex valued coefficient $z_{i,j}(t,f)$ on the $i$th trial at each time point and for each frequency. At any time point, these coefficients can be visualized as points or vectors in the complex plane, specified by their magnitude and phase. In the prestimulus period, ongoing activity is characterized by coefficients whose magnitudes and phases are randomly distributed and over many trials will form a cloud centered at the origin with zero mean and a uniform distribution of phases. The radius of the cloud, which is related to its variance, represents the amplitude of ongoing activity for that frequency component at that time point. A sensory stimulus may alter this ongoing activity, and thus this cloud of coefficients, in three ways. First, evoked activity describes new (“added”) activity at a particular frequency that is time-locked to the stimulus onset. Evoked activity is represented in the complex plane as a shift of the complex coefficients in the direction of a vector representing the additive component’s amplitude and phase (i.e., its angle with the horizontal axis). Second, an increase or decrease in the magnitude of the frequency component that is not time-locked to the stimulus (i.e., a change in variance) corresponds to reduced synchrony or desynchronization of activity. Induced activity will change the variance of the cloud but will not shift its center away from origin. Note that the terms “synchronization” and “desynchronization” suggest a simple reorganization of extant neuronal activity but could also involve an increase or decrease in activity levels (i.e., more or fewer neurons participating) as long as those changes are not time-locked to the stimulus. Finally, if the stimulus does not evoke additional activity but instead reorganizes the ongoing activity in the cortical network, i.e., triggers a phase reset, the coefficients will have nonrandom phase, but the magnitude of the distance from the origin to each point will be unchanged from the prestimulus condition. The stimulus may trigger evoked, induced and/or phase reset responses simultaneously.

Among the several ways we will measure the average properties of these complex coefficients, there are two that are distinct but easily confused. The complex mean ($\text{mean}_c$) is the mean of all the points in the cloud, taking into account their directions from the origin. Stimulus-related changes in the mean are usually associated with additive response components, but the mean$_c$ can also be changed by phase reset. Synchronization and desynchronization (i.e., increases and decreases in the variance of complex coefficients, variance$_c$) refer to amplitude changes that are phase invariant and have no effect on the mean$_c$. By contrast, the power of the coefficients is their magnitude squared, regardless of their phase, and is represented by the average of single-trial power spectrograms. The power is equal to the variance$_c$ plus the square of the magnitude of the mean$_c$. Thus both additive and
synchronizing/desynchronizing effects will appear as changes in the power spectrum, because in both cases the magnitude of the complex coefficients will change, whereas phase resets will not affect power (because any increases in the magnitude of the mean_τ are offset by decreases in the variance_τ). The analysis utilized here is intended to better distinguish between the three mechanisms that could mediate changes in the mean_τ and the power spectrum. It should be noted that while the three mechanisms proposed are independent, their measures (as discussed in the following paragraphs) are not. In RESULTS, “Disentangling effects on mean_τ, variance_τ, and phase_STM,” we estimate the contribution of this interdependence to the observed measures.

The three measures of interest, i.e., mean_τ, variance_τ, and phase_STM, are defined (Martinez-Montes et al. 2008) as

\[
\text{mean}_τ(t,f) = \frac{1}{L} \sum_{i=1}^{L} z_i(t,f)
\]

\[
\text{variance}_τ(t,f) = \frac{1}{L} \sum_{i=1}^{L} \left[ z_i(t,f) - \text{mean}_τ(t,f) \right]^2
\]

\[
\text{phase}_\text{STM}(t,f) = \frac{1}{L} \sum_{i=1}^{L} \left[ z_i(t,f) - \text{mean}_τ(t,f) \right] / \left[ z_i(t,f) - \text{mean}_τ(t,f) \right]
\]

where \( L \) is the number of trials. The mean_τ and variance_τ measures are standard forms; when plotting data, it was often more convenient to plot the square root of the variance_τ (i.e., standard deviation), to which we will refer as \( \text{var}_τ^2 \). The phase_STM measure merits some additional comments. This measure, the second trigonometric moment, or STM (Krieger et al. 2011), tries to capture any phase concentrations that may occur as a result of a stimulus and attempts to correct for the possibility that phase concentration is due to an additive shift in the complex coefficients by first subtracting the mean_τ and then measuring the uniformity of phase values such that a bimodal distribution results in high values of the measure and a uniform distribution results in low values.

To determine the effects of the stimulus on the mean_τ variance_τ, and phase_STM measures, we followed the procedure of Martinez-Montes et al. (2008) and computed “t-like” statistics that compare the value of these measures at any particular point in the time-frequency plane with the prestimulus distribution in a comparison window \((t = -550 \text{ to } -125 \text{ ms})\) as follows:

\[
t\text{-mean}_τ(t,f) = \sqrt{N - 1} \left[ \text{mean}_τ(t,f) - \mu_{\text{mean}}(f) \right] / \sigma_{\text{mean}}(f)
\]

where \( \mu_{\text{mean}}(f) \) and \( \sigma_{\text{mean}}(f) \) are the mean and standard deviation of \( \text{mean}_τ(t,f) \) computed across trials over the time window spanning the comparison region and \( N \) is the number of points in the comparison region. The t-variance_τ and t-phase_STM statistics are computed in an analogous manner. These statistics are called t-like because they do not follow the \( t \)-distribution, even though they are computed similarly. Because the distribution of these statistics is not known a priori, we tested the significance of these effects by comparing the computed \( t \)-statistics with a null distribution computed using data from the prestimulus period. The major problem with testing the significance of effects on coefficients in the time-frequency plane is correcting for multiple comparisons; there are many hundreds or thousands depending on the time and frequency resolution. We used a procedure similar to that of Martinez-Montes et al. (2008) and computed the local false discovery rate (FDR; Efron 2004), i.e., the ratio of the expected distribution (the null distribution normalized to the number of observations) to the data distribution, with a threshold criterion of \( q < 0.1 \), i.e., bins in the data distribution in which there are >10 times as many points as there are in the expected distribution were assumed to be significant. The value of 0.1 is the upper limit on the false positive rate yielded by the analysis over all bins. In practice, this rate is much lower and is obtained by dividing the tail area of the expected distribution by the tail area of the data distribution, with the tail defined as all bins larger or equal to the smallest significant bin on the right side and smaller than or equal to the largest significant bin on the left side. Here, these rates varied from 0.0034 to 0.030, with a median of 0.011. Note that wavelet and STFT coefficients were analyzed separately, because their time resolution was different. (For an alternative approach to statistical analysis of time-frequency data, see Kilner et al. 2005).

To obtain the null distribution, we computed the \( t \)-like statistics on the prestimulus data, smoothed this distribution using a Gaussian filter with \( s = 2 \) bins, and computed the expected distribution by scaling this estimate of the null to match the area of the similarly smoothed data distribution (i.e., the \( t \)-like statistics obtained from the poststimulus data; the portion of the data used for scaling were the bins corresponding to the central 98% of the null distribution). We note that our implementation of the local FDR analysis diverges from that of Efron and Martinez-Montes et al. in our choice of the null distribution. In the original description of this technique, the null distribution was obtained by fitting the middle part of the data distribution, (i.e., the distribution not including the tails) under the assumption that the “uninteresting” data points are normally distributed and that the “interesting” data points are only located in the tails. This method is highly dependent on the assumed distribution (e.g., an assumption of normality) and the range of data used to fit the chosen distribution. Fitting to a narrow range of data is likely to result in poor estimates of the null distribution, particularly if the null diverges from the assumed distribution, and fits to a large range of data may inadvertently incorporate “interesting” data points into the null. By contrast, we know a priori that the prestimulus baseline period of our data consists entirely of “uninteresting” points and thus provides an excellent estimate for the null distribution under the relatively benign assumption that the statistical properties of the null distribution do not change over the \( \sim 2 \text{-s} \) recording window. Our method also does not require assumptions about the shape of the null distribution, which is unknown for the \( t \)-like statistics. We note, however, that this choice is nonstandard and has not been rigorously tested. We also note that different, independent methods for determining statistically significant response components across our data set (see below) gave very similar results (Fig. 6), indicating that the method is sound at least in this context.

To pool data across animals, we compared two methods. In the first, we calculated for each animal the significance at each time-frequency point of changes in the mean_τ variance_τ, and phase_STM according to the local FDR method described above and then calculated the fraction of animals exhibiting significant changes at each time-frequency point (for variance_τ and phase_STM, we accounted for the sign of the change by coding in each animal at each time-frequency point a significant decrease as \(-1 \) and a significant increase as \(+1 \), adding up these values across animals, and then dividing by the number of animals). In the second method, we computed the mean_τ variance_τ, and phase_STM measures across trials as described above and then tested for significant changes from baseline using the Mann-Whitney U-test. Before significance testing, mean_τ and variance_τ measures were normalized at each frequency to \( \text{var}_τ^2 \) of the ongoing activity at that frequency. We used two correction methods for multiple comparisons: the local FDR method described above and the unweighted Bonferroni correction (i.e., the significance level is adjusted by dividing by the number of simultaneous tests, here the number of points in the time-frequency plane). The two methods yielded very similar results, differing qualitatively only for the phase_STM measure. Because the Bonferroni method strictly minimizes type I errors (i.e., false positives) at the expense of statistical power, it is considered to be an extremely conservative correction (Shaffer 1995). By contrast, FDR methods are designed to maximize statistical power while setting less stringent control on type I errors (Efron 2004). Thus it is likely that these two methods provide an upper and lower bound.
on the occurrence of significant stimulus-related changes in the measures of interest.

All electrophysiological data were analyzed using software written in Matlab v7.11 (The MathWorks, Natick, MA).

**Simulations**

We used simulations to determine the extent to which trial-by-trial variability in the amplitude and latency of an additive component could account for the observed changes in variance. Simulations were specific to each experiment, using ongoing activity and the evoked response from that experiment. For each experiment, we first estimated the spectrum of the ongoing activity just prior to the stimulus by averaging the wavelet transform and STFT data over the time period $-0.5 < t < -0.1$ s, where the stimulus onset occurred at $t = 0$ s. Next, for each trial, we simulated ongoing activity by adding sinusoidal components (frequency resolution $= 1$ Hz, $1 < f < f_{\text{Nyquist}}$) with phases uniformly distributed over $(0, 2\pi)$ and amplitudes normally distributed over $(\mu = 1, \sigma^2 = 0.5)$ across frequencies and trials. The spectrum of this simulated ongoing activity was matched to the recorded ongoing activity by multiplying each frequency component by the corresponding magnitude in the ongoing spectrum. For the additive component, we took the average time-domain response from that experiment from $-0.1 < t < 0.5$ s and windowed it by a tapered cosine (Tukey) window with $\alpha = 0.05$. This additive component was then scaled by a normally distributed amplitude factor ($\mu_{\text{amp}} = 1, 0 < \sigma_{\text{amp}} < 2.4$) added at variable latency ($\mu_{\text{lat}} = 0, 0 < \sigma_{\text{lat}} < 3.0$ ms) to the ongoing activity on each trial. As expected, the simulated mean measure matched well the recorded mean measure. The variance measure was a function of $\sigma_{\text{amp}}$ and $\sigma_{\text{lat}}$. We compared the simulation results with the recorded data by finding the values for $\sigma_{\text{amp}}$ and $\sigma_{\text{lat}}$ that minimized the mean squared error between the normalized var for the simulated and recorded data. To simplify this calculation, we evaluated the var measure at one time point for each frequency, corresponding to the maximum of the mean measure during the peristimulus time window. Because the frequency components were logarithmically spaced, the error function was weighted by $1/f^{0.5}$.

Phase resets will appear as increases in the mean measure and will also affect the variance measure. We used simulations to evaluate the magnitude of this effect, essentially asking whether the changes in mean and variance could be due to a phase reset whose magnitude equaled the observed phase concentration (see Fig. 10). We simulated ongoing activity as complex coefficients having real and imaginary parts that are jointly normally distributed with common variance. To simulate a pure phase reset, the magnitudes of these coefficients were maintained but their phases concentrated to adhere to a von Mises distribution with variable standard deviation. We then computed the complex mean and variance of the phase-concentrated simulated data and created lookup tables to relate these mean and variance values to the phase measure (Fig. 10A). Finally, we took the median across animals and, using the lookup tables, computed the expected changes in the normalized mean and var measures that would occur if they were entirely due to a phase reset producing these phase values (Fig. 10B).

**RESULTS**

**Ongoing and Stimulus-Related Activity in the Time and Time-Frequency Domains**

Time-domain responses to acoustic stimuli averaged across trials were similar to epidurally recorded auditory responses reported previously in rats (Fig. 1A) (e.g., Lazar and Metherate 2003; Nakamura et al. 2011; Ruusuvirta et al. 1998), with a short latency component that peaked between 10 and 20 ms after stimulus onset, followed by a series of peaks and troughs lasting for several hundred milliseconds. As previously described for cortical sensory responses, we observed a large amount of trial-by-trial variability; in many cases, single-trial responses were only as large as or were even dwarfed by ongoing activity (Fig. 1A). As we describe below, this variability has implications for interpreting responses in terms of evoked and induced components. We used time-frequency analysis to investigate ongoing and stimulus-related activity in more detail.

Time-frequency spectra of single-trial responses (Fig. 1B) also exhibited considerable trial-by-trial variability. Mean spectrograms averaged across trials (Fig. 1C) illustrate that power in the
prestimulus period falls off rapidly with frequency, as expected, and suggest that sensory stimuli evoke a strong, broadband, transient response that terminates with stimulus offset (Fig. 1C). To help visualize these features, we averaged these spectrograms across specific time windows before and after stimulus onset to yield the average spectral content of ongoing and stimulus-related cortical signals (Fig. 2). Mean prestimulus spectra were similar in shape across animals but spanned an order of magnitude in overall power. Spectra from individual animals and spectra averaged across animals showed that ongoing activity rarely exhibited distinct peaks that would correspond to strong oscillations; instead, the power of ongoing activity fell off as \( \sim 1/f^2 \) (Fig. 2A, dashed line).

Power spectra averaged across trials and across several time windows during and following the stimulus (20-ms-wide windows centered at \( t = 25, 125, 250, \) and 500 ms, where stimulus onset is at \( t = 0 \) ms and offset is at \( t = 250 \) ms) are illustrated in Fig. 2A, ii–v; the power spectrum of ongoing activity averaged across animals is replotted in each panel for comparison (dotted line). The relative increase in power due to the stimulus was computed by taking the ratio of the mean spectrum in each time window to the mean ongoing spectrum (Fig. 2B). Relative to ongoing activity, auditory stimuli triggered a rapid increase in power that grew in magnitude with frequency and was particularly pronounced at \( \sim 20 \) and \( \sim 200 \) Hz (Fig. 2, Aii and B, thick solid line). Midway through and at the end of the stimulus, there was a relative increase in low-frequency components, whereas the high-frequency components had largely returned to baseline (Fig. 2, Aiii, Aiv, and B, dashed and dotted lines). A small relative decrease in power was observed following stimulus offset at \( \sim 10 \) Hz (Fig. 2B, dot-dashed line).

In general, however, analysis of relative increases in these power spectra cannot resolve the contribution of evoked vs. induced response components. Below, we will dissect these contributions using a more sensitive analysis method of complex spectral coefficients.

**Complex Coefficients in the Time-Frequency Domain**

As illustrated in Fig. 1, there was marked trial-by-trial variability in ongoing activity and the responses to acoustic stimuli. This variability can be visualized by plotting the magnitude and phase of each frequency component on successive trials in the complex plane. In Fig. 3, we show the values of complex coefficients of four frequency components (\( f = 100, 30, 10, \) and 3 Hz; 1st column) measured at a single time point (\( t = -0.25 \) s) during the prestimulus period and at specific times following stimulus onset (\( t = 0.025, 0.2, \) and 0.75 s; 2nd–4th columns) for all 664 trials from this animal (pooled across 7 recording sessions). In these plots, each point is the coefficient from one trial and one instant in time plotted in the complex plane. The magnitude of the frequency component is the distance of this point from the origin; the angle this vector forms with the positive real axis is the phase of the frequency component at that instant in time. The meanC of the coefficients across trials is indicated by the line emanating from the origin. During the prestimulus period (1st column), the coefficients have approximately zero mean (i.e., the clouds are centered at the origin). Changes in the meanC of the coefficients correspond to an additive component at constant frequency.
phase at that frequency, i.e., an evoked response. The variance\(^C\) of the coefficients across trials is indicated by the radius of the circle, which is centered at the mean\(^C\). Changes in the variance\(^C\) correspond to an increase or decrease in the magnitude of that frequency component at random phase, i.e., so-called induced response components. Transient changes in the mean\(^C\) and variance\(^C\) of the coefficients are evident following the stimulus, as indicated by shifts in the centers of the circles (i.e., changes in the mean\(^C\)) and changes in the radii of the circles (i.e., changes in variance\(^C\)). For example, at 10 Hz, there is a large shift in the mean\(^C\) following stimulus onset, with a small associated change in the variance\(^C\) (Fig. 3, 3rd row, 2nd column), whereas after stimulus offset a small decrease in variance\(^C\) (i.e., desynchronization) is apparent (Fig. 3, 3rd row, 4th column). Stimulus-triggered phase concentrations would appear as deviations from circularity of the data cloud at particular angles relative to the positive real axis. One example is the first poststimulus time point for the 10-Hz component (Fig. 3, 3rd row, 2nd column). It is evident from these plots (i.e., changes in the mean\(^C\)) and changes in variance\(^C\)). For example, at 10 Hz, there is a large shift in the mean\(^C\) following stimulus onset, with a small associated change in the variance\(^C\) (Fig. 3, 3rd row, 2nd column), whereas after stimulus offset a small decrease in variance\(^C\) (i.e., desynchronization) is apparent (Fig. 3, 3rd row, 4th column). Stimulus-triggered phase concentrations would appear as deviations from circularity of the data cloud at particular angles relative to the positive real axis. One example is the first poststimulus time point for the 10-Hz component (Fig. 3, 3rd row, 2nd column). It is evident from these plots that both the mean\(^C\) and variance\(^C\) of the data can be affected by the stimulus but 2) that they also can be affected independently and 3) that strong phase concentrations, i.e., noncircularity of the data clouds, occur only occasionally and transiently. These three points are illustrated more clearly in Fig. 4, which plots the magnitude of the mean\(^C\), \(\text{var}^{1/2}\), and \(\text{phase}_{\text{STM}}\) measures averaged across trials for these four frequency components as a function of time relative to stimulus onset. In this example, changes in mean\(^C\) and variance\(^C\) are tightly linked during the stimulus (0 < \(t\) < 0.25 s), although for the 3-Hz component the later changes in mean\(^C\) occur without concomitant changes in variance\(^C\) (Fig. 4D). Note also that changes in the phase\(_{\text{STM}}\) measure tended to occur near peak changes in the mean\(^C\).

**Statistical Analysis of Complex Coefficients**

To quantify changes in the distribution of complex coefficients and determine the time course of these changes relative to baseline, we compared the mean\(^C\), variance\(^C\), and phase\(_{\text{STM}}\) measures.
values with those recorded in the prestimulus period by calculating t-like statistics as described in MATERIALS AND METHODS. Because we do not know the distributions of these statistics a priori, we tested the significance of these changes by comparing the calculated distributions with a null distribution of t-values computed during the prestimulus period (Fig. 5A) using the local FDR measure (Efron 2004; Martinez-Montes et al. 2008). Histograms of t-mean$_C$ (Fig. 5A, left), t-variance$_C$ (Fig. 5A, middle), and t-phase$_{STM}$ (Fig. 5A, right) values from the null region are smoothed to create null distributions (dashed lines). We used these empirically derived null distributions to determine significance. The corresponding raw (bars) and smoothed (solid lines) histograms of t-values for the poststimulus data at one specific frequency (9 Hz) are shown in Fig. 5B, in which we also plot the expected distributions (dashed lines; see MATERIALS AND METHODS). The underlying assumption of the local FDR technique is that the interesting (i.e., significant) data points are those with t-values that are in the tails of the data distribution, i.e., t-values that are rarely observed in the null distribution. These “interesting” points are illustrated in Fig. 5B (black bars), and their t-values are indicated via color coding in the time-frequency contour plots in Fig. 5C. In this experiment, the auditory stimulus triggered a broadband increase in the mean$_C$ that for low frequencies lasted well beyond the stimulus offset (Fig. 5C, left). A broadband increase in variance$_C$ (synchronization) during the stimulus was also observed, which at lower frequencies was followed by an extended period of desynchronization (Fig. 5C, middle). Significant changes in phase$_{STM}$ were observed at lower frequencies during the stimulus (Fig. 5C, right). Note that the increases in variance$_C$ and phase$_{STM}$ during the stimulus tended to co-occur with increases in the mean$_C$. Below, we show evidence that these correlated (i.e., co-occurring and of the same sign) changes in mean$_C$, variance$_C$, and phase$_{STM}$ during the stimulus are likely the result of trial-by-trial variability in the additive (i.e., evoked) component, rather than reflecting independent modulation of ongoing activity or true phase resets, as they are often interpreted.

Summary Across Animals

The method illustrated in Fig. 5 for determining significance of stimulus-related effects on mean$_C$, variance$_C$, and phase$_{STM}$ is effective for a single experiment but leaves open the issue of summarizing data across experiments and subjects. Epidural signals can vary widely across subjects in terms of raw power (Fig. 2), potentially complicating statistical comparisons. We compared two approaches to summarizing data across subjects, illustrated in Fig. 6. In the first approach, we computed “significance maps” averaged across animals (Fig. 6A; see MATERIALS AND METHODS), representing the fraction of animals showing significant stimulus-related effects at each point in the time-frequency plane. [Note that for the variance$_C$ map, negative values indicate the fraction of animals showing a decrease in variance$_C$.] Key features of the stimulus-related activity are: 1) a broadband increase in the mean$_C$ and an offset response that persists for up to 500 ms at the lowest frequencies.
analyzed (Fig. 6A, left), 2) a broadband synchronization that is always shorter than the duration of the stimulus (Fig. 6A, middle), 3) a late desynchronization for frequencies <30 Hz that lasts for several hundred milliseconds after stimulus offset (Fig. 6A, middle), and 4) a phase concentration that is significant in a minority of animals and that is broadband and brief at high frequencies but progressively longer at frequencies <30 Hz (Fig. 6A, right). Note that using this analysis technique (and unlike our observations using the power spectra of Fig. 2), we are able to detect substantial effects on spectral coefficients after stimulus offset.

Presentation of data as in Fig. 6A demonstrates the location of significant changes in the time-frequency plane but is not sufficient for describing the magnitude of these effects, and it is not easy to compare the relative effects across frequencies or between measures using these plots. Therefore, in Fig. 6B we summarize the measures themselves across animals by plotting the grand median for all points in the time-frequency plane, and in Fig. 6, C–E, we present these measures after thresholding for significance across animals as tested using a Mann-Whitney U-test at each time-frequency point and corrected for multiple comparisons using local FDR (Fig. 6, C and E) or the much more conservative Bonferroni correction (Fig. 6D). Values at each frequency are normalized to the mean of var1/2 at that frequency during the prestimulus period for each experiment, and then the grand median at each time-frequency point was computed across animals. For the meanC measure (Fig. 6, B–E, left), a value of 1 indicates an additive component equal in power to the prestimulus ongoing activity. Peaks in the meanC measure occurred at ~155 Hz (latency ~10 ms), 50.1 Hz (~25 ms), 19.2 Hz (~35 ms), and 9.19 Hz (~65 ms). The persistent poststimulus meanC component detected in this analysis was less prominent than in the analysis of Fig. 6A, appearing only at <30 Hz and with a duration <150 ms. In the Fig. 6, B–E, middle, we plot the var1/2 measure normalized as for the meanC measure, and thus a value of 1 represents no change compared with the prestimulus ongoing activity. Changes in the varianceC during the stimulus were highly correlated with changes in the meanC (best seen in the expanded view of Fig. 6E; for example, compare the high-frequency peak >100 Hz, as well as the secondary peaks in the gamma and beta range). As in Fig. 6A, after stimulus offset there is a late and long-lasting desynchronization in the alpha frequency band (~10 Hz; Fig. 6, C and D, middle) that is not associated with any changes in the meanC. The phaseSTM measure (Fig. 6, B–E, right) can take on values ranging from 0 to 1 (and thus needs no normalization), where 1 represents perfect phase alignment and 0 represents uniform phase distribution. On average, we observed values of the phaseSTM measure that were at most ~0.15 (Fig. 6B, right) but were consistent enough across animals to be statistically significant using the local FDR correction (Fig. 6, C and E, right) but not the Bonferroni correction. As with the varianceC measure, these changes in the phaseSTM measure were highly correlated

Fig. 6. Summary across animals of stimulus-related effects on meanC, varianceC, and phaseSTM. A: for each animal, points in the time-frequency plane showing a significant increase in the meanC (left), varianceC (middle), or phaseSTM (right) measure were assigned a value of 1, and points showing a significant decrease were assigned a value of -1 (varianceC and phaseSTM only). These significance maps were then averaged across animals, giving a summary map that shows the fraction of animals exhibiting significant changes at each time-frequency point. B: grand median of meanC (left), var1/2 (middle), and phaseSTM (right). The meanC and var1/2 measures were normalized for each animal to the var1/2 averaged over the prestimulus period. C: the same as B, but thresholded for significance across animals as assessed using the Mann-Whitney U-test and corrected for multiple comparisons using local FDR. D: the same as C, but corrected for multiple comparisons using Bonferroni correction. E: same data from C plotted on an expanded time scale. Note the high correlation, during the peristimulus period, of the meanC and var1/2 measures.
Disentangling Effects on Mean, Variance, and Phase

Origin of stimulus-related changes in variance. When interpreting the data of Fig. 6, it is important to explore the potential confounds that may be introduced by the entanglement of the mean$_C$, variance$_C$, and phaseSTM measures. For example, two aspects of our data raise the possibility that the observed changes in variance$_C$ may be due to variability in the timing or amplitude of the additive component, rather than a modulation of ongoing activity expected of an “induced” response. These aspects relate to the nature of variance$_C$ changes that would be expected if these changes were due to timing and amplitude variability in the additive component and are illustrated in Fig. 7A. The result of such variability would be a distortion of the data clouds of spectral coefficients at each time-frequency point across trials in two different ways. Variability in the amplitude of an additive component will result in increased variance$_C$, i.e., a bulge in the cloud, in the direction of the mean$_C$ component vector. This effect is easily understood if one considers the extreme case in which on half the trials the additive component is zero, and on half the trials its amplitude is constant and nonzero (Fig. 7A, top). In this case, the data cloud would split into two subclouds, and the variance$_C$ across the whole cloud would be increased. Importantly, this variance$_C$ would be correlated with the amplitude of the additive component (Fig. 7A, top). (Note also that such variability would also lead to a phase concentration, and thus a change in the phaseSTM measure. This issue will be discussed in more detail below.) Variability in timing of an additive component would result in increased variance$_C$ in the orthogonal dimension. Again, this effect is easily illustrated by considering the extreme case in which on half the trials one latency (i.e., phase) is observed, and on the other half a second phase is observed, splitting the data into two subclouds at right angles to the direction of the additive component (Fig. 7A, bottom). Less extreme cases of amplitude and latency variability will lead to smaller effects on variance$_C$ (not shown), and it should be noted that simultaneous variability in latency and amplitude will have additive effects on the variance$_C$ but will mitigate apparent phase concentrations (due to a less distorted data cloud). We will return to this point below.

Previous work (David et al. 2006) has shown, on the basis of theoretical considerations, that amplitude and latency variability will have different effects on the mean$_C$ and variance$_C$ measures. Introducing trial-by-trial variability in the amplitude of the additive component without changing its average value will result in correlated increases in the variance$_C$ measure without decreases in stimulus-related changes in the mean$_C$ measure. By contrast, varying the latency of the additive component on each trial will change its average value, thus decreasing the changes in the mean$_C$ measure. However, this effect will be frequency dependent, with larger effects at higher frequencies (Fig. 7A, bottom): if the latency variability is frequency-independent (e.g., $\sigma = 3$ ms), then frequencies with periods comparable to this latency variability will be smeared out and their mean$_C$ reduced and variance$_C$ increased, whereas frequencies with periods much greater will be relatively unaffected. In this case, high-frequency components of the mean$_C$ measure will be transferred to the corresponding frequencies in the variance$_C$ measure. Our data suggest that both sources of variability contributed to stimulus related changes in variance$_C$.

When considering the observed changes in mean$_C$ and variance$_C$ illustrated in Fig. 6B, we note that these changes were quite similar in their duration and frequency range, as would be expected if the stimulus-related variance$_C$ changes were secondary to trial-by-trial variability in the amplitude of an additive component. Figure 7B summarizes the co-occurrence of

![Fig. 7. Evidence that variance$_C$ changes are secondary to trial-by-trial variability in the mean. A: illustration of effects of amplitude and latency variability on the variance$_C$ of complex coefficients. Complex coefficients were simulated with real and imaginary components ($z$) normally distributed with mean$_C = 0$ and variance$_C = 1$ (top left). In all panels, var$_{1/2}$ is indicated by the radius of the circle, and the mean$_C$ is indicated by the black line connecting the origin to the center of the circle. The var$_{1/2}$ of the original data cloud (top left) is indicated by the dashed circle. Top row shows effect of amplitude variability on the var$_{1/2}$ measure, and that it is a monotonically increasing function of the magnitude of the additive component ($|I_{\text{add}}|$). For these data, half the points are unchanged and half have a constant additive component with the magnitude indicated. Bottom row shows the effect of latency variability on the var$_{1/2}$ measure, and that it is a monotonically increasing function of the ratio of latency to 1/f. For these data, all points were summed with an additive component with constant amplitude, but for the 2 halves of the points the additive component had 2 different phase values, and the difference between the 2 phase values was set by the ratio of latency (3 ms) to 1/f multiplied by $\pi/4$. B: 2-dimensional (time-frequency) correlation coefficients between normalized mean$_C$ and var$_{1/2}$ computed over the peristimulus time window for STFT coefficients (i.e., $3 < f < 16$ Hz; left) and wavelet coefficients (i.e., $16 < f < 300$ Hz; right). Data from individual animals appear as open circles. Box plots denote median (horizontal line) and 1st and 3rd quartiles (box). Note the high correlation in most animals, as would be expected for variability in the amplitude of the additive component. C: median (thick line) and 1st and 3rd quartiles (thin lines) across animals of the normalized var$_{1/2}$, computed at each frequency at the time point corresponding to the peak in the normalized mean$_C$ (see MATERIALS AND METHODS). Note that the var$_{1/2}$ increases with frequency, as would be expected for variability in the latency of the additive component. Cross corr, cross-correlation.]}
meanC and varianceC changes as measured by the two-dimensional (i.e., time and frequency) cross-correlation of changes in meanC and varianceC over the peristimulus time window. For both STFT (f < 16 Hz) and wavelet coefficients (i.e., f > 16 Hz), we observed a significant correlation in all animals (median correlation coefficients = 0.68 and 0.59, respectively).

Although stimulus-related changes in meanC and varianceC tended to co-occur, leading to the correlation illustrated in Fig. 7B, the magnitude of changes in varianceC relative to simultaneous changes in meanC was greater for high- vs. low-frequency components, as would be expected if the underlying mechanism was variability in the latency of additive components (David et al. 2006). We quantified this effect by measuring var1/2 at one time point for each frequency, corresponding to the time of the peak of the change in the mean measure. The median normalized var1/2 across our data set is much larger for frequencies above about 100 Hz (Fig. 7C), an observation that is consistent with trial-by-trial variability in latency of the additive component contributing to the observed stimulus-related changes in varianceC (David et al. 2006).

To determine whether these two types of variability could account for the observed peristimulus changes in varianceC, we simulated the occurrence of only an additive component, which varied from trial to trial in amplitude and latency, and looked for the effects on the varianceC and phaseSTM measures. Simulations based on data from one experiment are illustrated in Fig. 8. From left to right, Fig. 8 shows the mean spectrogram, normalized meanC and var1/2 measures, and phaseSTM measure. The raw recorded data from this experiment (Fig. 8A) are typical, i.e., the changes in varianceC and meanC are highly correlated during the stimulus (2-dimensional correlation coefficient for the window 0 < t < 0.25 s is 0.73 for 3 < f < 16 Hz and 0.52 for 16 < f < 300 Hz), but the change in varianceC at high frequencies is much larger, relative to the meanC, compared with lower frequencies. As expected, when we simulate the ongoing activity with an invariant additive component (Fig. 8B), the mean spectrogram and normalized meanC measure exhibit an excellent fit to the recorded data (1st and 2nd columns), and with no variability included in the model there are no observed changes in varianceC and phaseSTM (3rd and 4th columns). We varied σamp and σlat systematically over a wide range of values and found the best fit of the model to the data by finding the parameter values that minimized the difference between the observed and simulated changes in normalized var1/2 measure (see MATERIALS AND METHODS). With σlat = 0, the best fit of the model occurred for σamp = 1.0 (i.e., equal to the mean amplitude; Fig. 8C). This amplitude variability successfully captured key features of the recorded varianceC changes, i.e., the simulated changes in varianceC measure were highly correlated with the changes in meanC, especially for f < 100 Hz. At higher frequencies, the magnitude of changes in the recorded varianceC measure was far greater than could be accounted for by amplitude variability alone. In addition, the amplitude variability caused an apparent phase concentration, as expected due to the elongation of the data cloud along the direction of the additive vector, and this phase concentration was somewhat larger than observed in the recorded data. When we varied both σamp (0.8) and σlat (1.8 ms), we could capture some of the “extra” varianceC at high frequencies (Fig. 8D), although the observed varianceC still exceeded that of the model, and because the latency variability caused an elongation in the data cloud in the orthogonal direction, the apparent phase concentration was reduced.

We determined the optimal model parameters by minimizing the difference between the recorded data and the model output over the normalized var1/2 as a function of frequency. The resulting functions for the experiment of Fig. 8, A–D, are shown in Fig. 8E. We repeated this analysis for each animal and found that the median across animals of the optimal variability was 0.9 (1st, 3rd quartiles = 0.7, 1.8) for σamp and 1.8 ms (1st, 3rd quartiles = 0.7, 2.0 ms) for σlat. Using these best fits, we computed the median of the normalized var1/2 as a function of frequency across animals (Fig. 8F). In both the single animal example in Fig. 8E and the summary across animals in Fig. 8F, we note that the model produced excellent fits for f < 100 Hz with σlat = 0 and improved fits for f > 100 Hz when σlat was also varied. The divergence of the fits for f > 100 Hz reflects a limitation of the model, which is based solely on the observed additive component. Introducing amplitude variability is straightforward; such variability will not affect the mean of the additive component. However, introducing latency variability is more complicated, as varying the latency of the additive component will alter its mean in a frequency-dependent manner (i.e., the additive component becomes smeared, and the smearing is worse for frequency components on the order of the inverse of the latency variability). Therefore, with this modeling approach, we are likely to underestimate the contribution of an additive component with variable latency to the observed varianceC at those high frequencies. Despite this limitation, the modeling results strongly suggest that broadband changes in varianceC that co-occur with changes in the meanC as well as high-frequency changes in varianceC that can occur even in the absence of changes in the meanC are all likely to reflect variability in amplitude and latency, respectively, of an additive component, rather than induced responses secondary to reorganization of ongoing activity or top-down modulation of network connectivity parameters.

To summarize these simulations across animals, we computed the median stimulus-related changes in normalized var1/2 and phaseSTM, as in Fig. 6B, from the best fits of the model for each experiment. This analysis is illustrated in Fig. 9, where we have also plotted the recorded data for comparison (Fig. 9, A and B; 1st column). Over the data set, the model with only amplitude variability was able to capture much of the changes in varianceC that were correlated with changes in the meanC (Fig. 9A, 2nd column), but in this case the changes in phaseSTM were far stronger than those in the recorded data (Fig. 9B, 2nd column). A modest improvement in model fit for varianceC and a substantial improvement in phaseSTM was observed with both amplitude and latency variability (Fig. 9, A and B, 3rd column). Although the phaseSTM values still exceeded those in the recorded data, subtracting the model fit from the data revealed that those differences were modest (Fig. 9B, 4th column). By subtracting the varianceC measure obtained from simulations from the recorded data (Fig. 9A, 4th column), we observe that nearly all the early varianceC changes <30 Hz, and most of these early changes up to 100 Hz, could be accounted for by the model with both amplitude and latency variability.

Effect of phase reset on stimulus-related changes in mean. As stated above, additive components can appear as phase concentrations, and the phaseSTM measure employed here was specifically designed to address this confound (at least in the
However, the complementary problem is not addressed: phase resets can still appear as changes in the mean [this problem is why phase resets can contribute to the time-domain mean response and have thus been postulated to underlie evoked responses (Makeig et al. 2002; Sayers et al. 1974)]. The conflation of phase reset and amplitude variability has also been observed in the context of relating amplitude variations and dynamic correlations in human magnetoencephalography (MEG) data (Friston et al. 1997). This effect is easily illustrated when considering the coef-
ficients in the complex plane. Beginning with a cloud of coefficients scattered about the origin (i.e., with zero mean), as in Fig. 3 (left column), changing the phases of those points so that they are all restricted to one quadrant will produce a data cloud with nonzero mean_C and altered variance_C (see Martínez-Montes et al. 2008 for illustrative figures). Thus it is possible in the data of Fig. 6 that some portion of the stimulus-related phase concentration (Fig. 6, right) may actually be due to a stimulus-related phase concentration (Fig. 6, left). Ideally, one would estimate the contribution of phase reset to the mean_C effect using the phaseSTM measure, i.e., by calculating how much of a change in the mean_C is expected from the observed phase concentration. However, because of the way the phaseSTM measure is defined, its values cannot be analytically translated into relative effects on the mean_C. Instead, we used simulations to estimate the changes in normalized mean_C and var^{1/2} expected if they were entirely due to phase resets whose magnitude is given by changes in the phaseSTM measure (see MATERIALS AND METHODS; Fig. 10, A and B). From these simulations, it is clear that phase resets can have nontrivial effects on the mean_C and variance_C of coefficients (Fig. 10B). However, when we compare these phase effects with the observed changes in mean_C and variance_C (Fig. 10C), we note two major discrepancies. First, the magnitude of predicted changes in the mean_C are far smaller than those observed (Fig. 10, B and C, left), and second, the simulated phase reset is associated with a decrease in variance_C, not the large increase that we observe (Fig. 10, B and C, middle). Thus, although we cannot rule out the possibility that the observed phaseSTM values represent true phase resets and contribute to the changes in mean_C and variance_C observed, these effects cannot account for all of the observed changes in the mean_C and would require a large compensatory increase in variance_C. As we illustrated above, a far more parsimonious explanation for the observed changes in mean_C, variance_C, and phaseSTM derives from variability in the additive component.

State Dependence of Response Variability

Previous reports have shown that cortical response variability is state dependent (Curto et al. 2009; Kisley and Gerstein 1999; Pasley et al. 2009; White et al. 2012), and in humans discriminability of acoustic stimuli depends on the level of ongoing activity in auditory cortex (Sadaghiani et al. 2009). Delta power is an indicator of level of arousal (Jung et al. 1997; Santamaria and Chiappa 1987), and sensory responses have been shown previously to depend on delta power and delta phase (Curto et al. 2009; Lakatos et al. 2005; Luo et al. 2010). We investigated whether the variability of the additive component depended on the state of the cortical network by comparing this variability for each animal with the ratio of gamma to delta power in the prestimulus period. Low values of this ratio are associated with lower levels of arousal (Curto et al. 2009; Kisley and Gerstein 1999; Pasley et al. 2009; White et al. 2012), and in humans discriminability of acoustic stimuli depends on the level of ongoing activity in auditory cortex (Sadaghiani et al. 2009). Delta power is an indicator of level of arousal (Jung et al. 1997; Santamaria and Chiappa 1987), and sensory responses have been shown previously to depend on delta power and delta phase (Curto et al. 2009; Lakatos et al. 2005; Luo et al. 2010). We investigated whether the variability of the additive component depended on the state of the cortical network by comparing this variability for each animal with the ratio of gamma to delta power in the prestimulus period. Low values of this ratio are associated with lower levels of arousal (Curto et al. 2009). Amplitude and latency variability for each animal was measured using the parameters \( \sigma_{\text{amp}} \) and \( \sigma_{\text{lat}} \) respectively, of the best model fit to the data. We found that \( \sigma_{\text{amp}} \) declined exponentially with gamma-to-delta ratio over the 16 recorded animals, and a linear fit to \( \sigma_{\text{amp}} \) vs. the log of this ratio yielded a significant negative correlation (Fig. 11A; slope = −0.78; \( r^2 = 0.38; P = 0.01 \)). No significant correlation was evident for \( \sigma_{\text{lat}} \) (not shown; \( m = 0.50; r^2 = 0.17; P = 0.11 \)).

The data of Fig. 11A indicate that animals exhibiting higher levels of cortical arousal had less variable additive components in their stimulus-related responses. Because the data were collected over a period of 0.5–1.5 h spread across multiple days, it is likely that cortical state varied over the recording period and even on a trial-by-trial basis. To obtain a single-trial measure of response variability, we calculated the Euclidean distance between single-trial responses and the mean response...
Thus it is possible that some of the effect observed in additive component and variability in the additive component the response due to ongoing activity summing with an invariant delta oscillations on trials in which gamma-to-delta ratio was

PhaseSTM value are also shown (C, assuming the changes were entirely due to phase reset. Data were obtained in 14 of 16 animals (Fig. 11C), which may include effects of ongoing delta power) and the analysis of Fig. 11D (which may lack state-dependent effects on the variability of the delta component of the response).

**DISCUSSION**

**Summary and Conclusions**

By analyzing the mean, variance, and phase of complex spectral coefficients of cortical neural activity in rats, we have demonstrated that auditory stimuli lead to broadband changes in all three measures that are onset-dominated and tend to co-occur. In the peristimulus response window, the high correlation in these measures in the time-frequency plane is consistent with a simple model in which an evoked response component is elicited on each trial with variable amplitude and latency. Simulations of trial-by-trial variability using this model account for most of the observed changes in varianceC and PhaseSTM without engaging any nonlinear, “top-down” changes in ongoing activity typically associated with induced responses or true phase resets. The only unequivocal induced response component we detected in our data set was a long-latency, long-lasting desynchronization in the alpha band that was modest but consistent across animals. Using simulations and single-trial measures of variability in our recorded data, we have shown that this variability was most pronounced when ongoing cortical activity indicated the lowest levels of cortical arousal. These observations are consistent with auditory cortical responses in these passive listening conditions being dominated by a variable, state-dependent, “bottom-up” information stream, with only modest, long-lasting changes in cortical network activity.

**Variability in Amplitude and Latency of Additive Components**

The contribution of amplitude and latency variability to apparent induced responses has been noted previously (David et al. 2006). The classical model of evoked response generation consists of an additive component with fixed latency and amplitude that linearly combines with variable ongoing activity to produce single-trial responses. According to this model, averaging can reliably recover the invariant additive component, and subtraction of this mean evoked response from single trials will reveal interactions between the evoked response and ongoing activity that comprise the induced response. However,
it is now well established that this model can result in a poor estimate of the evoked response, whose amplitude and latency can vary considerably from trial to trial (D’Avanzo et al. 2011; Lange et al. 1997; Mocks et al. 1987; Truccolo et al. 2002). Nevertheless, the most common method for computing the induced response component continues to be subtraction of the time-domain evoked response from single-trial responses (e.g., Crone et al. 2001; Steinschneider et al. 2008; Trautner et al. 2006). Our data show that caution must be used when interpreting induced responses, particularly when they co-occur with evoked responses, and that trial-by-trial variability in latency and amplitude can account for a significant portion of observed increases in variance of spectral coefficients.

**Contribution of Phase Reset to Stimulus-Related Activity**

The contribution to evoked responses of phase reset vs. additive components continues to be hotly debated, and previous studies in humans investigating the origin of the evoked response and the contribution of phase reset have reached contradictory conclusions (Hanslmayr et al. 2007; Krieg et al. 2011; Jervis et al. 1983; Makeig et al. 2002; Makinen et al. 2005; Sauseng et al. 2007; Sayers et al. 1974). Detailed analyses and simulations have shown that it is often difficult to conclude unequivocally that observed phase concentrations are due to phase resets per se rather than an additive component (Krieg et al. 2011; Sauseng et al. 2007). The phaseSTM measure employed here, in which the meanC is subtracted before the phase concentration is calculated (Martinez-Montes et al. 2008), was developed specifically to address this confound and performs well in the case of an invariant additive component (Krieg et al. 2011). However, our simulations show that variability in the amplitude of the additive component will also lead to a phase concentration, because this variability elongates the cloud of spectral coefficients in the complex plane along the axis of the meanC component and thus would be detected as a phase concentration even after the averaged meanC component is subtracted. Indeed, such an effect can be pronounced (e.g., Fig. 9B, 2nd column). Adding to the model variability in the latency of the additive component will also reduce the apparent phase concentration. Importantly, these simulations illustrate that a phase concentration exceeding that observed in our recorded data (Fig. 9) can be produced solely through variability in the amplitude and latency of an additive component. Because there is strong evidence that such variability is occurring in the animals recorded here in response to acoustic stimuli, it is not necessary to invoke a true phase reset of ongoing activity to account for the observed phase concentration.

A few studies that pay careful attention to these confounds have presented some evidence for the contribution of phase reset to cortical sensory responses (Krieg et al. 2011), but in

![Graph](http://jn.physiology.org/)

**Fig. 11.** Cortical state dependence of amplitude variability. A: amplitude variability ($\sigma_{amp}$) from best fit of model to observed var$^{1/2}$ for each animal as a function of the log of the ratio of gamma to delta power in the prestimulus period averaged over all trials for that animal. Solid line is linear regression fit to data (slope = −0.78, $r^2 = 0.38, P = 0.01$). Note that amplitude variability was greatest in animals with small gamma-to-delta ratios. B: single-trial analysis for all trials recorded in 1 animal. For each trial, we calculated the Euclidean distance between that single-trial response and the mean response for this animal. These single-trial distance measures are plotted as a function of log (gamma-to-delta ratio). Straight line is linear regression fit to the data (slope = −0.27, $r^2 = 0.14, P = 1.1 \times 10^{-14}$). Circles are the same data after shuffling trial number 100 times. C and D: slopes (circles) and 95% confidence intervals (vertical lines) obtained from linear regression fits as in B for all 16 animals without additional high-pass filtering (C) and after filtering $\geq 4$ Hz (D). Note that before filtering, 14 of 16 animals exhibit significant regression slopes, and after filtering, significant slopes are shown in 8 of 16 animals.
auditory cortex such responses appear to be limited to integration with stimuli from other modalities (Lakatos et al. 2007; Luo et al. 2010). This is consistent with our results that phase reset contributes little to auditory cortical responses under passive listening conditions in the frequency range examined (3–300 Hz). However, in our recordings, we also observed very little structure in the frequency content of ongoing activity (Fig. 2), and it is possible that phase reset of ongoing components may contribute more to the evoked response when there is more structure to the ongoing activity (Sauseng et al. 2007) or during dynamic naturalistic stimuli (Ng et al. 2013). Finally, it should be noted that although phase concentration due to an additive component is not the same as a phase reset of ongoing activity and implies primarily bottom-up influences rather than modulation of ongoing activity, the end result of both mechanisms is an alignment or phase synchronization that could equivalently facilitate integration within the cortical network.

We note here that the opposite confound can also occur, i.e., a phase reset can also cause apparent changes in the mean and variance (Fig. 10), as has been reported previously (Krieg et al. 2011; Sauseng et al. 2007), and an intuitive understanding of this effect can also be gained by considering the cloud of spectral coefficients in the complex plane. However, we have used simulations of phase reset to show that resulting changes in the mean and variance are smaller than (in the case of the mean) or are in the opposite direction of (in the case of the variance) the changes observed in our recordings, and thus a phase reset is unlikely to contribute to the changes in mean and variance reported here.

Spectral Content of Ongoing Activity

In our recordings, we observed little evidence for peaks in power spectra derived from ongoing activity (Fig. 2). In one previous study on auditory cortex in monkeys, peaks in the delta, theta, and gamma bands were suggestive of consistent oscillations in these frequency ranges, and stimulus-triggered phase resets of these oscillations are reported to contribute to the response (Lakatos et al. 2005). Other studies did not find such peaks in ongoing activity (Brosch et al. 2002), indicating that such structure could be state dependent. It is unclear, however, what bearing such peaks have on the relationship between ongoing activity and stimulus-related responses. For example, a dominant peak in the power spectrum may not be necessary for phase reset at that frequency to occur (Sauseng et al. 2007). The presence of induced response components may similarly be unconnected to such peaks. For example, we observed evidence for stimulus-related alpha band desynchronization (Fig. 6) even in the absence of a prominent alpha peak in the spectrum of ongoing activity (Fig. 2), and induced oscillatory activity in the gamma band has been observed in the absence of detectable gamma peaks in the ongoing spectrum (Brosch et al. 2002). Thus the presence or absence of “peaks” in the ongoing spectrum may not be indicative of the network’s capacity for stimulus-induced reorganization of oscillatory activity, perhaps because of the local nature of these rhythms or modulation of specific dynamic components of the network in a stimulus-specific manner.

Alpha Band Desynchronization

We observed desynchronization centered in the alpha band around 10 Hz (Fig. 6, A–D, middle). Cortical alpha band desynchronization is well known in human studies of visual, somatosensory, and auditory sensory processing to reflect arousal and attentional mechanisms (for recent reviews, see Foote and Snyder 2011; Palva and Palva 2007; Weisz et al. 2011). The desynchronization we observed in the alpha band is similar to what has been observed in human auditory cortex (Crone et al. 2001; Lehtela et al. 1997; Palva and Palva 2007), although other studies report increases in alpha power in response to acoustic stimulation (Karrasch et al. 1998; Krause et al. 1996; Steinschneider et al. 2008). Interestingly, there have been few reports of alpha desynchronization in auditory cortex of animals, although this response has been observed in other sensory cortices (Sobolewski et al. 2011; Wiest and Nicolelis 2003). It is possible that the modest reduction in variance that we observed here is common and may have remained undetected in some studies. In human subjects, alpha desynchronization coincides with increased excitation in the cortical circuit during information processing (Klimesch et al. 2007; Weisz et al. 2011), i.e., alpha power is positively correlated with local inhibitory tone and is postulated to reflect “behavioral idling” that is suppressed during enhanced information flow through the thalamo-cortical network (Sobolewski et al. 2011). Thus, according to this model, the long-lasting desynchronization reflects a long-lasting rebound excitation (Qin et al. 2007; Recanzone et al. 2000), in parallel with a similar rebound or persistent excitation indicated by the post-stimulus change in mean. If the desynchronization does indeed reflect changes in inhibitory network activity, the observation that these changes are not time-locked to the stimulus may mean that changes in inhibitory tone are governed by intracortical connections, e.g., top-down inputs (Klimesch et al. 2007).

Gamma Response Components

In sensory cortex, changes in gamma band (30–120 Hz) power and in gamma frequency synchronization across cortical regions have been linked to feature binding, object representation, perception, and memory of meaningful stimuli in humans (Pulvermüller et al. 1996; Tallon-Baudry and Bertrand 1999). In auditory cortex, gamma band activity evoked by acoustic stimuli typically has a short latency, evoked (i.e., phase-locked) component (Galambos et al. 1981; Pantel et al. 1991), as well as both short latency and prolonged induced components that are poorly phase-locked and can outlast the stimulus duration by hundreds of milliseconds (Crone et al. 2001; Brosch et al. 2002; Jeschke et al. 2008; Lenz et al. 2008; MacDonald and Barth 1995; Steinschneider et al. 2008; Tallon-Baudry and Bertrand 1999). In our recordings, we observed stimulus-related activity throughout the gamma range, but our data and simulations indicate that these responses were dominated by evoked response components. This result is in contrast to results obtained during discrimination tasks in humans and rodents (Crone et al. 2001; Jeschke et al. 2008; Lenz et al. 2008), in which “induced” gamma band responses were observed and whose power could correlate with memory and sensory processes. The differences between these results and those reported presently could be ascribed to the passive
listening conditions under which we recorded our data, although high gamma induced responses have also been reported for passive listening conditions in monkeys (Steinschneider et al. 2008). It should be noted that in these studies no attempt was made to distinguish between changes in \( \text{variance}_C \) associated with amplitude and latency variability in an additive component vs. changes in \( \text{variance}_C \) due to reorganization of ongoing activity or top-down modulation of cortical network connectivity. Indeed, although the latency of the induced high gamma activity in the latter study (~50 ms) was longer than in our recordings, it was striking that these effects were concentrated in the highest frequency components, consistent with these “induced” components being due to latency variability in the evoked component.

State Dependence of Variability and Functional Implications

It is widely recognized that cortical responses exhibit substantial trial-by-trial variability (de Kock et al. 2007; Luczak et al. 2013; Shadlen and Newsome 1998; Whitsett et al. 1977). However, the origin of this variability is still under debate, with some reports arguing that cortical responses can be modeled as a constant additive component combined with variable ongoing activity (Arieli et al. 1996; Dawson 1954; Risner et al. 2009; Shah et al. 2004), whereas others demonstrate that models including variable response components provide superior fits to the data (Truccolo et al. 2002). The difference between these models depends on the relative importance of local variable ongoing activity in the cortical network itself, as opposed to variability in ascending input to the network. Our data are consistent with these latter models and provide strong evidence that under passive listening conditions, stimulus-related responses in auditory cortex of rats are dominated by an evoked component with variable amplitude and latency, consistent with variability in the ascending input to auditory cortex. This trial-by-trial variability is not surprising given the probabilistic nature of neuronal responses throughout the ascending sensory pathway. Although responses of cortical cells tend to be more variable than their thalamic inputs, the latter still exhibit substantial trial-by-trial variability (de Kock et al. 2007) and in the ascending auditory pathway, information content, reflecting in part variability in responses, is comparable between thalamus and cortex (Chechik et al. 2006). Understanding the source of this variability and how it changes with behavioral state will yield insight into the state dependence of sensory processing and the neural basis of sensory awareness.

We present evidence that the variability of stimulus-related activity depends on the state of the cortical network (Fig. 11). Using two different measures, we find that the amplitude variability of the additive component is smallest when the ratio of gamma to delta power is high. Because the latter is commonly used as a metric of arousal, this result suggests that arousal is associated with reduced response variability. We note that since variability was highest when the animals were most quiescent, it is unlikely to derive from trial-by-trial variability in positioning of the animals’ heads relative to the speakers. The intertrial interval employed here is far longer than is typical for electrophysiological studies of the auditory system, in which intervals of 1 s or less are not uncommon. These long intervals likely reduce the predictability of the stimulus time and thus may reveal more strongly endogenous sources of response variability. Previous reports have demonstrated such state dependence of response variability. For example, the variability of cortical responses is reduced over the course of perceptual learning (Adab and Vogels 2011) and exhibits dose dependence under general anesthesia (Kisley and Gerstein 1999). Attention also reduces variability and is associated with improved behavioral performance (Ledberg et al. 2012). However, in some reports spontaneous cortical activity is reduced on average during slow-wave sleep (Nir et al. 2012; Steriade et al. 1978), when delta power is high, and this would likely reduce response variability, contrary to our findings that variability is maximal when delta power is low. Analyses such as those presented here that can identify this variability and how it changes with learning or awareness will prove useful tools for understanding the underlying mechanisms of these state changes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.M.K. and M.I.B. analyzed data; B.M.K. and M.I.B. interpreted results of experiments; B.M.K. and M.I.B. prepared figures; B.M.K. and M.I.B. drafted manuscript; B.M.K. edited and revised manuscript; B.M.K. and M.I.B. approved final version of manuscript; M.I.B. conception and design of research; M.I.B. performed experiments.

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