Dynamics of Ongoing Activity: Explanation of the Large Variability in Evoked Cortical Responses

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Evoked activity in the mammalian cortex and the resulting behavioral responses exhibit a large variability to repeated presentations of the same stimulus. This study examined whether the variability can be attributed to ongoing activity. Ongoing and evoked spatiotemporal activity patterns in the cat visual cortex were measured with real-time optical imaging; local field potentials and discharges of single neurons were recorded simultaneously, by electrophysiological techniques. The evoked activity appeared deterministic, and the variability resulted from the dynamics of ongoing activity, presumably reflecting the instantaneous state of cortical networks. In spite of the large variability, evoked responses in single trials could be predicted by linear summation of the deterministic response and the preceding ongoing activity. Ongoing activity must play an important role in cortical function and cannot be ignored in exploration of cognitive processes.

When a stimulus is presented repeatedly, the variability of the evoked cortical responses is often as large as the response itself, both in anesthetized (*1*) and in awake, behaving animals (*2*). The standard approach has been to adopt a "signal-plus-noise" model, assuming that an individual evoked response is composed of a reproducible signal added to uncorrelated noise. The signal is then recovered experimentally from the noise by averaging over repeated trials (*3*). This approach tacitly assumes that variability reflects "noise," which is a nuisance for cortical processing and could be overcome by the brain by appropriate averaging over populations of neurons (*4*). Numerous articles deal with the question of what the source of variability in the brain is (*5*, *6*). This issue of the reliability of cortical responses must be resolved in order to determine whether the neural code for information transfer in the brain requires the averaged activity of many neurons (*7*).

Ongoing cortical activity is far from being just noise (*8*). In fact, the spontaneous activity of a single neuron is not an independent process but is time-locked to the firing or to the synaptic inputs from numerous other neurons, all activated in a coherent fashion, even without sensory input. Often the coherent ongoing activity is as large as evoked activity. Therefore, ongoing activity must have a major influence on sensory processing. We present evidence for the hypothesis that cortical evoked activity comprises a reproducible stimulus response and a dynamically changing ongoing activity, presumably reflecting varying brain states (*9*).

We tested the above hypothesis by analyzing the spatiotemporal dynamics in single-trial responses to visual stimulation (moving gratings). Experiments were carried out on six anesthetized, muscle-relaxed adult cats as described elsewhere (*8*, *10*). Activity was measured in the visual cortex (areas 17 and 18), combining real-time optical imaging and electrophysiological recordings. A 2-mm-square area of primary visual cortex, stained with the voltage-sensitive dye RH795, was imaged onto a 12 × 12 array of photodiodes. Simultaneously, spike discharges of two isolated neurons and the local field potential (LFP) were recorded from a microelectrode inserted into the exposed area. Optical and electrical signals were continuously sampled every 3.5 ms for periods of 70 s.

Real-time optical imaging with the use of voltage-sensitive dyes measures, at millisecond time resolution, the membrane potential changes of populations of neuron processes (*11*). It emphasizes synaptic input, and hence, the signal is similar to the LFP (*8*, *12*). We analyzed the dynamics of the nonaveraged activities in single trials and their organization in space and time (*13*, *14*). This analysis enabled us to assess the extent to which individual cortical response patterns are influenced by the instantaneous network state. Optically recorded images together with traces of the simultaneously recorded LFP and spike trains are shown in Fig. 1A for two responses to a repeated visual stimulus. The large variability revealed in the optically imaged responses resembles the well-known variability in the LFP and single-neuron recordings. The fact that the response variability of synaptic population activity, measured optically and in LFP, is at least as large as the response itself argues against the assumption that averaging over local neuron populations would eliminate response variability (*4*). Averaging over trials (Fig. 1B) does remove this variability and extracts the reproducible re-

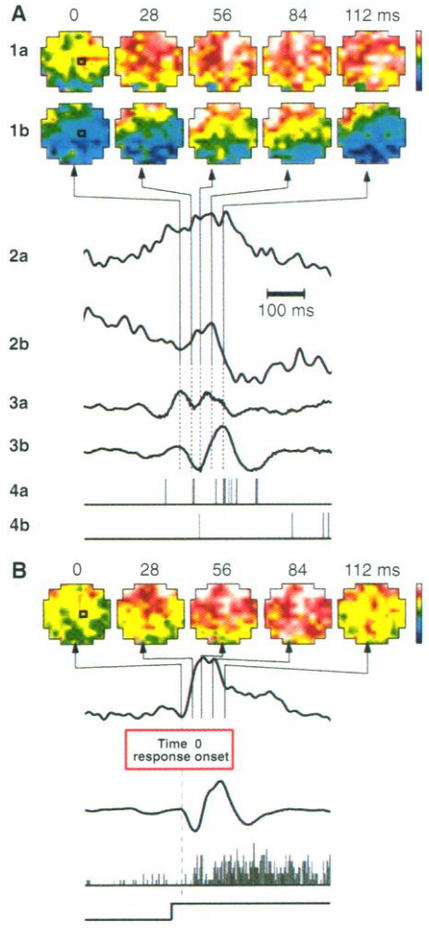

Fig. 1. Evoked activity in response to repetitive stimulation exhibits large variability. (**A**) Two individual responses (a and b) to a repeated visual stimulus [bottom trace in (B)]: The images (1a,b) show the activity in a 2 mm by 2 mm area of cortex, taken at different times from response onset. Activation above the mean level is coded in red, suppression in blue, as indicated by the color scale (right); full scale corresponds to a fractional change of $\sim 5 \times 10^{-5}$). The small square in the first image marks the site, above the microelectrode, from which the optical traces (2a,b) were taken. Note the large variability in the evoked response, also reflected in the LFP (3a,b) and single-neuron spike trains (4a,b), both recorded simultaneously with the optical signals. The absence of slow components in the LFP is due to high-pass filtering above 3 Hz. (**B**) Average evoked response: The optical images and signals, LFP, and single-unit activity were averaged, triggered on the onset of 34 visual stimuli (drifting full-field grating) in the preferred orientation of the recorded unit.

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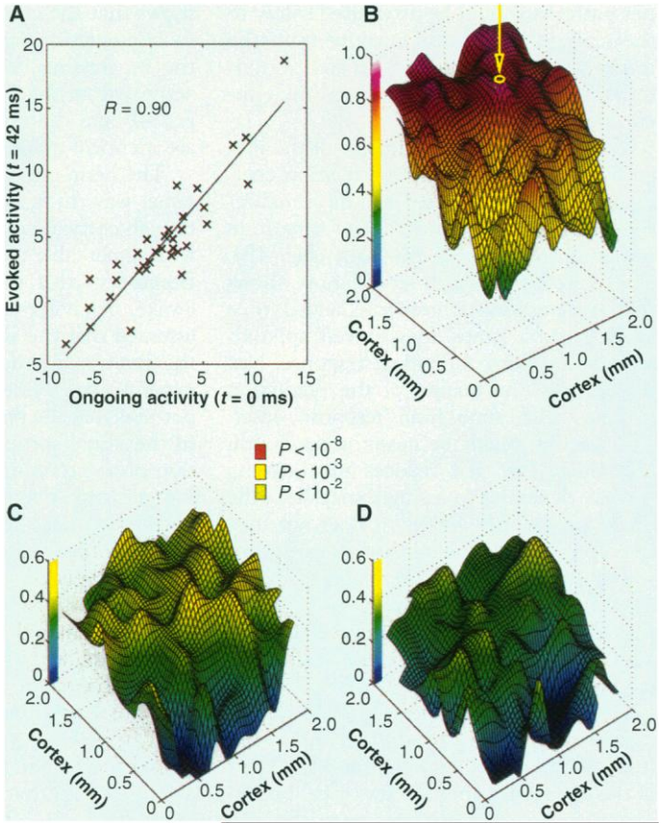

Fig. 2. Cortical evoked activity is related to the initial state. (**A**) Scatter plot of optically measured evoked activity at a single cortical site 42 ms after response onset in 34 successive single trials versus the initial state at that site. Both axes have the same arbitrary units. The straight line depicts the result of linear regression (correlation coefficient $R = 0.9$). (**B**) Correlation coefficients [as in (A)] for all sites in the imaged cortical area. The arrow marks the site, selected in (A). The statistical significance of correlation is indicated by color. (**C**) Correlation between the evoked LFP 28 ms after response onset and the initial state. (**D**) Correlation between the evoked spike rate, measured over an interval of 35 ms centered around 28 ms after response onset, and the initial state. The correlations in (C) and (D) are between a single site (microelectrode recording) and all optically measured sites.

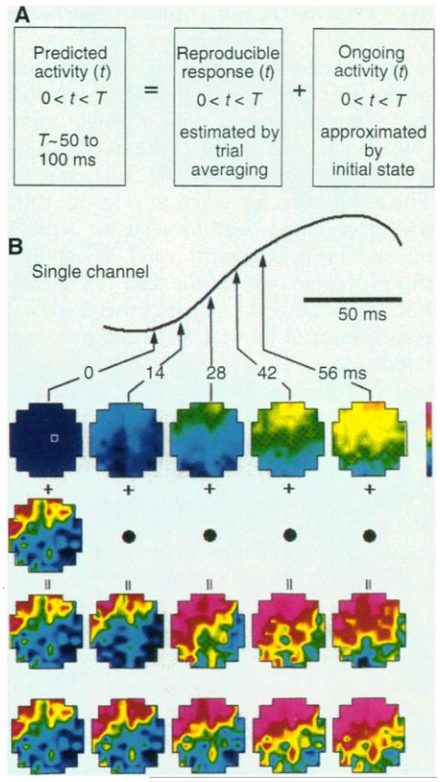

Fig. 3. Predicting the cortical evoked response. (**A**) A single-trial response to a stimulus was predicted by summing the reproducible response and the ongoing activity, approximated by the initial state. (**B**) Comparison of the predicted and measured responses. (Top trace) Averaged evoked response (34 trials), measured from a single optical channel above the microelectrode site (small square in top-left frame). (First row) Averaged evoked activity pattern (after subtraction of frame 0), shown at five different times after response onset, indicated by the arrows. All other rows show single-trial responses. (Second row) Initial state, approximating ongoing activity during the response. (Third row) Predicted response, obtained by adding the frames in the first and second rows. (Fourth row) Measured response.

sponse. We define time 0 as the moment just before the onset of the average response. The optically measured activity pattern at time 0 in an individual trial is here referred to as the initial state of that trial.

Searching for systematic rules underlying the response variability, we found that the evoked activity is highly correlated to the initial state: The evoked activity is low when the initial state was low, whereas it is high when the initial state was high. The relation between the two is approximately linear (Fig. 2A), as expressed by the high correlation coefficient ($R = 0.9$, $P < 10^{-12}$, $n = 34$ trials). Such high correlation was found for most of the recorded area (Fig. 2B) ($P < 0.001$ in all 35 recording sessions from six cats, each session containing 34 trials). The correlation was not restricted to the optical recordings, but held for the electrophysiological recordings as well. Indeed, the initial state was significantly correlated over a large area with the evoked LFP (Fig. 2C) [$P < 0.01$ in 89% (31/35) of the sessions] and, albeit to a lower extent, with the single-neuron spike rate (Fig. 2D) [$P < 0.01$ in 69% (24/35) of the sessions]. The correlation across the different types of electrophysiological recordings is expected to be considerably smaller because they reflect different aspects of cortical activity and different resolutions in space and time. The optical signal reflects localized changes in membrane potential, emphasizing synaptic input restricted to the upper cortical layers. On the other hand, the LFP reflects the extracellular currents near the electrode tip, with an ambiguous relation between the amplitude and polarity of the LFP waves and the brain cell activity in the vicinity of the microelectrode (*15*). In the simplest approximation, the LFP is the derivative of the optical signal. However, both signals are continuous waves that reflect the activity of thousands of neurons and are correlated to the state of the animal (*16*). The action potentials (spikes), with a time resolution of milliseconds, reflect the output of single neurons rather than of a population. In view of these considerations, our findings exhibit a remarkable consistency across cortical activities at greatly different spatial resolutions, measured by very different recording techniques.

The high correlations observed in single trials are consistent with the assumption that the stimulus-evoked activity contains a reproducible response component and that the changes in the patterns of evoked activity from trial to trial are caused by the fluctuating ongoing activity. This view is expressed in a simplified model (Fig. 3A) in which an individual response is the sum of two components: the reproducible response and the ongoing activity. Thus, the effect of a stimulus might be likened to the additional ripples caused by tossing a stone into a wavy sea.

A consequence of this simplified model is that we should be able to predict the response pattern in a single trial by taking into account the initial state of that trial. This prediction should hold for as long as the ongoing activity pattern (which presumably continues to change during the evoked response) is still similar to the initial state. Given that most of the energy in

the LFP is restricted to frequencies below about 20 Hz, we expect our prediction to perform well for up to 50 ms after response onset. We calculated the predicted response by adding the initial state, a single frame (Fig. 3B, second row), to the averaged response, a series of frames (Fig. 3B, first row). The result of such prediction (Fig. 3B, third row) corresponds well to what we actually measured (Fig. 3B, fourth row). We applied this procedure to all of the data (1190 trials from six cats) and compared the predicted responses, trial by trial, with the measured responses.

Particularly good examples of the prediction are shown in Fig. 4A for three consecutive trials in a recording session, examining the images obtained 28 ms after response onset. Note that the predictions for different trials vary only in their initial states. The variability among these initial states (first column) is so large and the patterns are so heterogeneous that the evoked activity in single trials (second column) looks very different each time. Yet, in all of these cases we obtained excellent predictions of the evoked activity pattern (third column), in spite of the large variability. Such good predictions were obtained for many of our trials, for periods of tens of milliseconds after response onset. Subtracting the initial state (first column) from the measured response (second column) leaves a net pattern ([M − I], last column): a single-trial estimate of the reproducible response to this particular stimulus. These net patterns are very similar, whereas the measured patterns (second column) are variable, suggesting that "removal" of the ongoing activity from the measured response does markedly reduce the response variability. We do not know if the lack of a perfect match among the net patterns should be attributed solely to the change of ongoing activity from the initial state or whether, in addition, it reflects deviations from the simplified, linear model.

To quantify the performance of the prediction, we measured the correlation coefficient between predicted and measured response patterns as a function of time from response onset for all the data (Fig. 4B). The long-lasting high correlation shows that a deterministic response added to a varying initial state does indeed approximate the varying individual response. Not surprisingly, the quality of the prediction declines with time from response onset. This decline occurs because the prediction procedure (Fig. 3B) reduces the ongoing activity dynamics to a single snapshot (the initial state). Specifically, it does not take into account that the ongoing activity continues to change while the evoked response unfolds. Evidently, we cannot directly measure the ongoing pattern during that time. We could estimate the expected time course of this change, however, by determining the autocorrelation of the optically measured activity patterns, triggered on the response onset (Fig. 4C). The left-hand part of the graph describes the statistical behavior of the ongoing activity up to the moment of response onset, and the right-hand part shows the statistical behavior of the activity after the initiation of the response. Clearly, the background ongoing activity has a very similar time course to the evoked activity (the evoked activity lasted for ~100 ms). In fact, the remarkable similarity between the two halves of the graph indicates that, on average, the ongoing dynamics are not affected by the response. The excellent resemblance between the curve in Fig. 4B and the left-hand part of Fig. 4C shows that the gradual decline in the quality of prediction can indeed be attributed to the progressing deviation of the ongoing activity from the initial state (the curve in Fig. 4B and the right-hand part of Fig. 4C are identical mathematically).

The brain often does not respond in the same way to a repeated stimulus, even though cortical neurons are able to respond with remarkable temporal accuracy (*5*, *17*). Because of this variability, found also in awake, behaving monkeys (*2*), it has been assumed that the signal is contaminated by the brain's "noise." Our findings provide experimental evidence to support the hypothesis that the processing of sensory input in the visual cortex involves the combination of a deterministic response and ongoing network dynamics. The relation between ongoing activity and evoked response in first approximation is linear (*18*). The combination of these components accounts for the large response variability in individual trials. It is well established that the ongoing activity measured by the electroencephalogram (EEG) is correlated to behavioral state and cognitive processes (*16*). In previous work (*8*, *19*), we characterized the ongoing activity measured optically, showing that it is strongly correlated with the local EEG and is composed of highly structured, ever-changing patterns of coherent activity. Taken together, these findings indicate that old notions of what is "noise" in brain activity may have to be revised. Because the ongoing activity is often very large, we would expect it to play a major role in cortical function. It may provide the neuronal substrate for the dependence of sensory information processing on context and on behavioral and conscious states. Indeed, the ongoing activity also affects the behavior of the awake macaque

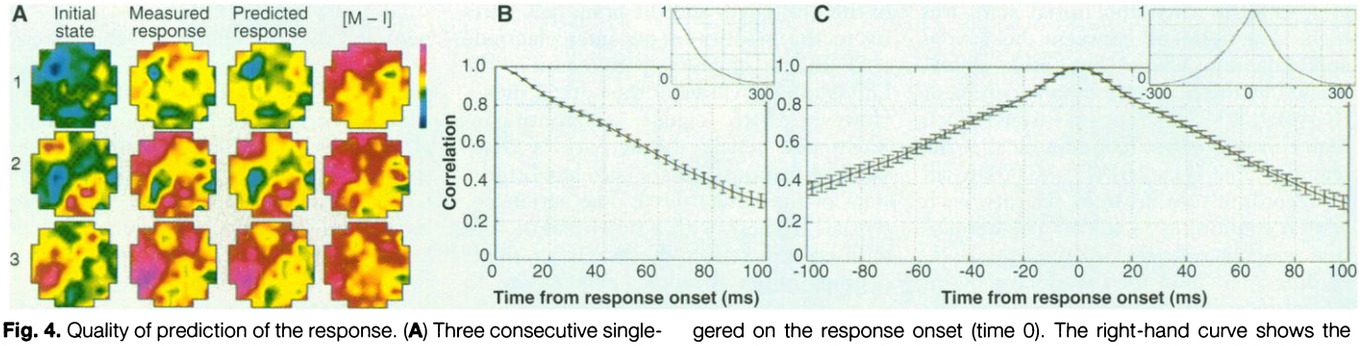

Fig. 4. Quality of prediction of the response. (**A**) Three consecutive single-trial responses (1 though 3) to the same visual stimulus, showing the initial state, the measured response 28 ms later, and the predicted response at that time. Subtracting the initial state from the measured response yielded the net pattern [M − I]. (**B**) Quality of prediction, assessed by the correlation coefficient between predicted and optically measured activity patterns as a function of time from response onset. The curve shows the mean correlation; the error bars denote the standard error of the mean (n = 35 recording sessions). (**C**) Autocorrelation of optically measured activity patterns, triggered on the response onset (time 0). The right-hand curve shows the correlation coefficient between the ongoing activity at time 0 (just before response onset) and the evoked activity. The left-hand curve shows the correlation coefficient between the same ongoing activity at time 0 and the ongoing activity before stimulus onset. After calculating the correlation coefficient for each pixel in the matrix at a certain delay, we simply summed all the pixels (because we did not see any consistent temporal differences between the different pixels). The insets in (B) and (C) show the correlations over prolonged time.

monkey: The reaction time in an arm-reaching paradigm could be predicted from the ongoing activity preceding the arm movement (*20*).

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Temporal Hierarchical Control of Singing in Birds

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Songs of birds comprise hierarchical sets of vocal gestures. In zebra finches, songs include notes and syllables (groups of notes) delivered in fixed sequences. During singing, premotor neurons in the forebrain nucleus HVc exhibited reliable changes in activity rates whose patterns were uniquely associated with syllable identity. Neurons in the forebrain nucleus robustus archistriatalis, which receives input from the HVc, exhibited precisely timed and structured bursts of activity that were uniquely associated with note identity. Hence, units of vocal behavior are represented hierarchically in the avian forebrain. The representation of temporal sequences at each level of the hierarchy may be established by means of a decoding process involving interactions of higher level input with intrinsic local circuitry. Behavior is apparently represented by precise temporal patterning of spike trains at lower levels of the hierarchy.

The neural codes that define discrete units of episodic behavior and organize these units into temporal sequences are not well established. Vocalizations constitute a group of behaviors for which correct temporal sequencing of discrete, often stereotyped events is fundamental to proper execution (*1*). Participation of midbrain structures in the generation of simple calls is well known in both mammals and birds (*2*). Less is known about the contribution of forebrain structures, particularly in the production of more complex vocalizations such as human speech and bird songs. Here, we characterize singing-related neuronal activity in the nuclei HVc and robustus archistriatalis (RA) of the zebra finch (*Taeniopygia guttata*). We present evidence for the hierarchical organization of neural codes that corresponds to the hierarchical organization of the singing behavior.

Zebra finch songs are hierarchically organized vocalizations formed by discrete acoustic elements (syllables) separated by intervals of silence (*3*). Song syllables can be classified into distinct classes (types) on the basis of acoustic features. Each syllable, in turn, can be further divided into acoustically distinct notes. The typical zebra finch song begins with a variable number of identical, simple introductory syllables comprising one or two notes, followed by a fixed sequence (motif) of multinote syllables. The motifs are repeated in longer versions of songs and are often separated by introductory syllables or other simple "connecting" syllables.

We developed techniques to record single-unit and multiple-unit neuronal activity in the HVc and RA of singing adult male zebra finches (*4*). Multiple sites were recorded in each nucleus in several birds who were good singers, resulting in a large database of vocalizations and associated neuronal activities [94 ± 92 (mean ± SD) songs per bird, $n = 13$ birds]. The onset and offset time and the identity of each syllable and note were established manually or by an automatic technique (*5*) whose output was verified manually. This procedure was essential for veridical analysis because the exact timing of the sequence of song elements varied from song to song. Additional long records (300 s) of ongoing activity

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