Time delays in the β/γ cycle operate on the level of individual neurons

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Recent studies suggested that small time delays among synchronized responses can convey information about visual stimuli. We compared these delays across different types of synchronized signals: single-unit action potentials, multi-unit action potentials, and local field potentials obtained with invasive recordings from cat visual cortex and magnetoencephalographic and electroencephalographic signals recorded from the scalp of human beings. In the signals that reflected more localized sources, time delays were larger and more selective for stimulus properties than in the signals that reflected more large-scale sources. The results suggest that a cortical code for stimulus features that exploits time delays operates predominantly across individual neurons rather than across larger anatomical structures such as brain areas. *NeuroReport* **21:746–750 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Neurons are said to be synchronized when they generate action potentials simultaneously more often than expected by chance. Neural synchrony, which is supported by oscillations in the β/γ band, has been investigated with different methods and experimental paradigms in numerous species and was found to be associated with cognitive and executive functions [1,2]. Recently, it has been suggested that synchrony needs not to be perfect (i.e. with zero-delays) but small delays between the discharges of synchronized neurons may provide computational advantages, containing information about the stimuli [3–7]. These studies investigated single-unit activity and multi-unit activity (SUA and MUA), but not other, more global signals, as reflected by local field potentials (LFP), magnetoencephalography (MEG) and electroencephalography (EEG).

To investigate temporal patterns created by delays in synchronization across different scales, we compared five different brain signals that reflect different levels of aggregation of neuronal activity: SUA, MUA, LFP, MEG and EEG. The first three were recorded invasively from anesthetized cats and the latter two from human participants performing a cognitive task. For each type of recording we applied the same analysis methods [4,7] to investigate the relative delays between the signals synchronized in the β/γ frequency band.

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Methods

Recordings and data preprocessing

In the experiments for collecting the SUA, MUA and LFP signals, six cats were anesthetized and visually stimulated with moving gratings. The electrophysiological signals were collected with Michigan probes inserted in area 17. In four cats, SUA, MUA and LFP were collected simultaneously (from the same electrodes). In one cat, only LFP and MUA were collected simultaneously. Finally, recordings from another cat were used only for the SUA analysis. For the data from one cat, the orientation tunings of MUA were reported in [6]. No other data were published before.

Human participants were instructed to perform a visual discrimination task either of Mooney faces (MEG, 13 participants, the same paradigm as the EEG recordings in [8]) or of simple geometrical objects formed by collinearly arranged Gabor patches (EEG, 11 participants). MEG and EEG signals were recorded from 266 and 64 sites, respectively, of which only those collected from the occipital areas were used in the analysis (seven and eight sites, respectively), as the methods required that all pairs of sites (electrodes) synchronize strongly enough in the β/γ range to yield accurate fits of Gaussian functions in cross-correlograms.

Although the visual stimuli for the different recording techniques differed, each stimulus was expected to be optimal for the activation of the brain area involved in the analysis. Further details on stimulus properties and

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recording techniques can be found in Supplementary Methods (Supplemental digital content 3 *http://links.lww.com/WNR/A55)*.

Cross-correlation and relative activation times

Cross-correlograms were computed between pairs of nodes, which could be single units, multi units, LFP signals (electrodes), MEG sensors or EEG electrodes. To attenuate the contributions of components slower than 20 Hz, we used a scaled correlation analysis with a scale of 50 ms (Nikolić, Singer and Mureşan, personal communication). The resulting cross-correlograms were averaged across trials and fitted by a Gaussian function to extract the preferred time delays between the pairs of nodes [7]. If, for a given stimulus condition, the fit was poor ($r^2 < 0.5$) in at least 50% of the pairs, this condition was excluded from the analysis. The same criterion was used to exclude nodes. These criteria permitted the analysis of 7–12 units in 2–12 stimulus conditions for SUA, 7–8 channels in 3–6 stimulus conditions for the MUA and LFP signals, seven channels in two stimulus conditions for the MEG signals and eight channels in three stimulus conditions for the EEG signals. The complex networks of pair-wise delays were transformed into simple representations of one-dimensional sequences [4]. The position in each sequence indicates the timing of a given signal of one node relative to all the others, and can be interpreted as the preferred temporal position of that signal within a cycle of the β/γ oscillation. In the following text, we refer to these temporal positions as relative activation times.

Statistical analysis

To evaluate the temporal spread of the relative activation times within each sequence, we estimated the total span between the earliest and latest activation times, ρ, and, separately, the standard deviation of activation times, σ, across all the members of the sequence. To estimate the degree to which relative activation times changed as a function of stimulus condition, we calculated a parameter κ, as follows: For each pair of stimulus conditions, the change in the relative activation time, expressed in milliseconds, was computed first for each node and κ was then defined as the mean square of the stimulus-induced changes across all the nodes of a given network. The changes represented by κ include random noise in addition to the internal adjustments of the relative activation times. Hence, a corrected κ' was computed by using the estimate of the variability caused by noise, which was obtained from a split of the data into odd and even trials:

$$\kappa' = \kappa \times \frac{\kappa_{stim}}{\kappa_{stim} + \kappa_{noise}}$$

in which κ_{stim} represents stimulus-induced and κ_{noise}, noise-induced changes. Stimulus-induced changes were first computed for odd and even trials separately and then averaged. κ_{noise} is the average of noise-induced changes indicated by the differences in estimates across odd and even trials.

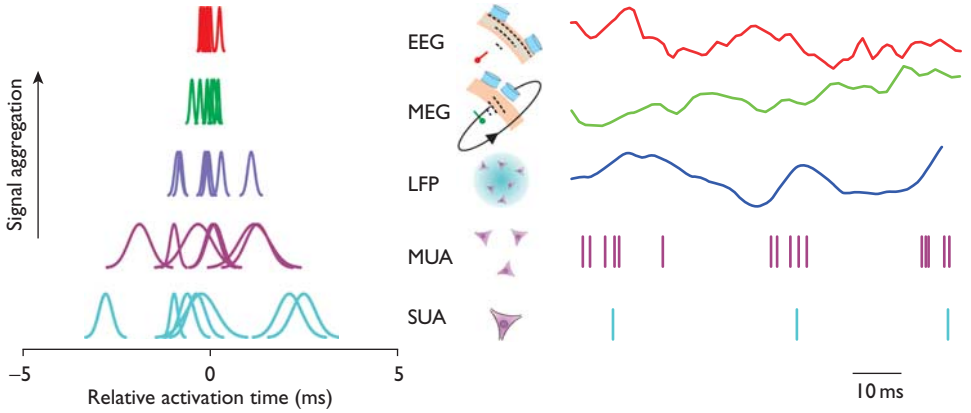
To avoid type-I errors in statistical inference because of multiple comparisons, ρ and σ values were averaged across all stimulus conditions and κ values across all pairs of stimulus conditions. To make legitimate comparisons across species and recording methods, the statistical analyses (t-tests) of ρ, σ and κ values were made always across participants (i.e. $N = 5$ cats for SUA, MUA, LFP signals; $N = 13$ and $N = 11$ participants for MEG and EEG signals, respectively). In addition, t-tests were computed only between the signals reflecting neighboring scales of integration (i.e. SUA–MUA, MUA–LFP, LFP–MEG, MEG–EEG). Each MUA could be paired to a corresponding LFP signal recorded from the same electrode and hence, for these comparisons, we used t-tests for paired measurements (i.e. repeated measurement design). All other comparisons were made with unpaired t-tests.

Results

The magnitudes and the total spans of relative activation times differed markedly across signal types. The time spans were largest for SUA (on average 7.26 ms) and gradually decreased with increasing globality of the recorded signal, the smallest being for the EEG signal (on an average 0.36 ms) (see Fig. 1 for example activation sequence of each type). Thus, the hierarchy of the magnitudes of delays matched the hierarchy of the spatial resolution of the signals. The higher the spatial resolution, the larger were the relative delays. More details are shown in Fig. 2. The paired comparisons between the activation time of the MUA and LFP signals in all conditions and cats are shown in Figs 2a and b. Both, the total span, ρ, and the standard deviation, σ, were consistently higher for MUA than for LFPs. For all the five types of signals, these values are summarized in Figs 2c and d. Significant differences in the covered time spans, ρ, and their standard deviations, σ, were found for all comparisons between the neighboring levels of spatial resolution except for the MEG and EEG signals, despite the trend. See Supplementary Tables 1 (supplemental digital content 1 *http://links.lww.com/WNR/A53* and supplemental digital content 2 *http://links.lww.com/WNR/A54*) for details on statistical analyses.

If relative activation times play a functional role in information processing, they should change systematically as a function of stimulus properties [4]. As would be expected from the magnitudes of the total time spans, stimulus-dependent changes in relative activation times were larger for the local than the global signals. As an example, in Fig. 3a, the changes in the activation times are compared for the paired LFP and MUA signals. The same stimuli induced much larger changes in the

Fig. 1



The magnitudes of relative activation times typically observed in the different types of neurophysiological signals. Left: delays in synchronization between the different types of signals expressed as relative activation times. The following abbreviations are used: EEG, electroencephalogram; LFP, local field potential; MEG, megnetoencephalogram; MUA, multi-unit spiking activity; SUA: single-unit spiking activity. The peak of each Gaussian on the time axis denotes the relative activation time of a given unit. The width of each Gaussian indicates the measurement error. Middle and right: the origin of each signal type and example traces.

activation times for MUA than for the LFP signals. Quantification of such changes, indicated by κ and κ', showed a hierarchy consistent with that reported above for the delay spans. The more global signals exhibited smaller changes in activation times. For the pair-wise comparisons of stimulus-dependent changes in activation times across different scales, significant differences were observed between MUA and LFP but not between SUA and MUA, and between MEG and EEG but not between LFP and MEG. Supplementary Tables 1 and 2 (Supplemental digital content 1 *http://links.lww.com/WNR/A53* and Supplemental digital content 2 *http://links.lww.com/WNR/A54)* provide further details.

Discussion

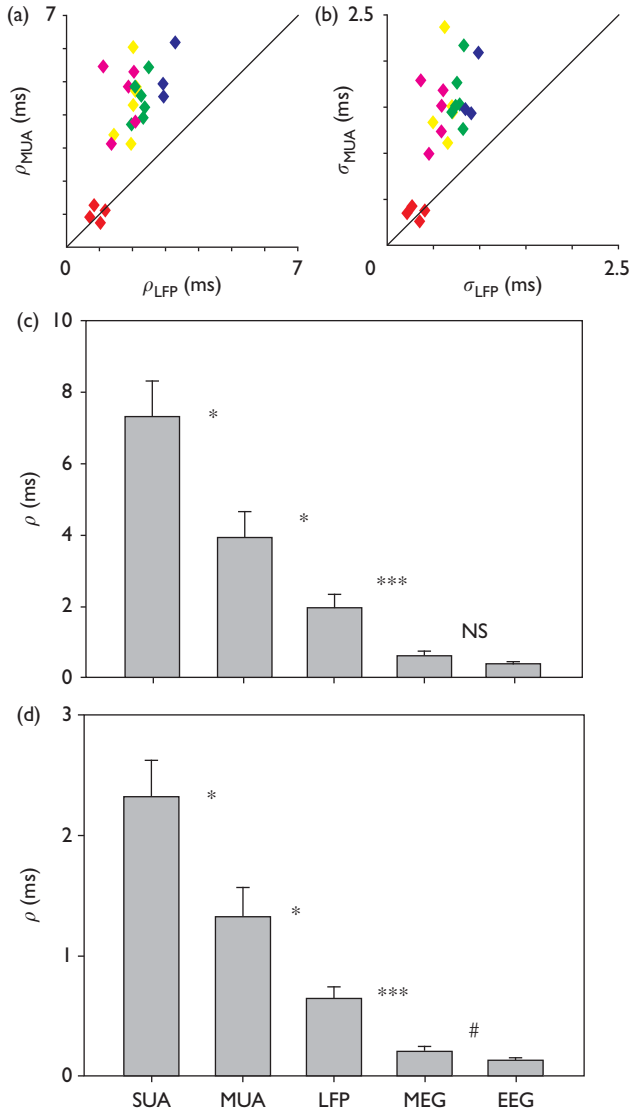
In this study, we compared relative time delays in neuronal signals evoked by visual stimuli and recorded at five different spatial scales (SUA, MUA, LFP, MEG and EEG), wherein we combined the recordings from anesthetized cats and human participants engaged in cognitive tasks. We found that both the magnitude of the delays and their stimulus-dependent changes varied consistently with the spatial scale of the signal; Signals with higher spatial resolution yielded larger delays and larger stimulus-dependent changes. This strong correlation between the spatial and temporal resolution of the signals suggested that the coding mechanism of the relative activation times operates at the level of individual neurons.

We compared the discrete responses (SUA and MUA) with continuous signals (LFP, MEG and EEG) but consider it unlikely, however, that this had an influence on the results. First, we applied mathematically equivalent methods for the computations of cross-correlograms and identical tools for all subsequent analyses. Second, we found similar results irrespective of whether the comparisons are made within or between the two groups of signals.

A more important caveat for the interpretation of this data is related to the fact that they were obtained from two different species with brains of greatly different sizes that were in radically different states (anesthetized and attentive, respectively), and exposed to rather different visual stimuli. This is not likely to affect the conclusions drawn from each of the different data sets nor is it expected to invalidate the comparisons between SUA, MUA and LFPs, and MEG and EEG, respectively, but it requires more careful comparisons between cat and human data. The results obtained in the cats show a significant positive correlation between the spatial scale and the temporal resolution of the signals. This trend is also found in the human data and in the comparisons between the cat and human results. Nevertheless, the best experimental design would be to combine SUA, MUA, LFP and surface EEG in the same animal.

These results provide novel insights into the organization of cortical networks and allow one to make predictions on the type of information extractable from the various signals. The results on the stimulus dependence of the SUA and MUA responses confirm that the relative timing of spikes in a synchronized cluster of discharges contains stimulus-related information [3–7]. This information is greatly reduced in the LFP signals, indicating that the spatial scale at which the stimulus-specific activation sequences of individual neurons are organized (i.e. firing sequences) is finer grained than the spatial resolution of the LFPs, which reflect the summed activity of neuronal profiles within a radius of up to approximately 250 μm [9].

Fig. 2



The magnitudes of relative activation times differ between the signal types. (a) Comparisons of the total spans, ρ, of relative activation times for the two signal types for which we could make direct pair-wise comparisons, that is, each MUA had a corresponding LFP. Each color denotes data obtained from one cat (five cats in total), and each data point is obtained for another stimulation condition (three to six). (b) The same analysis as in (a) but made for standard deviation, σ. (c) Comparisons of the total spans, ρ, of the relative activation times between all the five signal types. (d) The same analysis as in (c) but made for standard deviation, σ. NS, non-significant; #marginally significant value, $P=0.096$; *$P<0.05$; ***$P<0.001$.

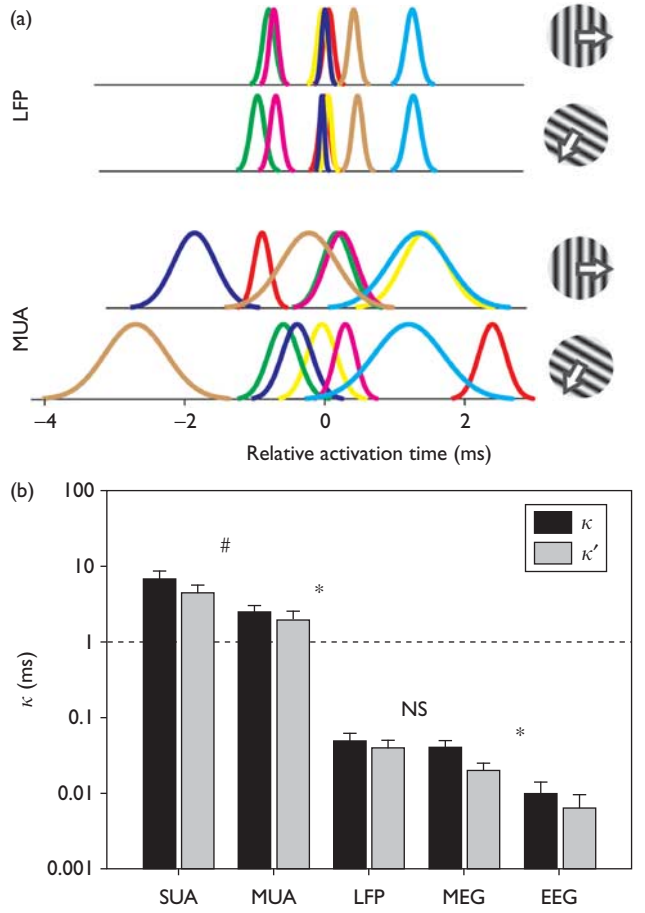
Thus, the information containing the adjustment of relative activation times seems to operate at the level of individual neurons or small groups of neurons, and the resulting shifts in the relative firing times tend to average out among the group of cells contributing to the LFP. As the close correlations among the MUA and LFP signals show [10], the two types of signals can be used nearly interchangeably to assess amplitude and time course of synchronization and oscillatory patterning of cell groups within volumes corresponding approximately to a functional column in the visual cortex. Accordingly, recordings of LFPs and more global signals cannot be used to retrieve the information encoded in the relative timing of spikes within the cluster discharges, which are often synchronized with LFP oscillations. The function of LFP may be similar to a carrier rhythm, that is, a temporal reference that the neurons use to adjust their relative firing times [11].

Interestingly, multisite recordings have shown that phase angles between the LFP and EEG signals recorded from different sites within or across cortical areas can be substantial [12–14] and can change in a task-dependent manner [15–18]. Theoretical arguments [19] and experimental evidence [12] suggest that these phase adjustments serve to dynamically modulate the functional coupling between anatomically connected cells. These data are not in disagreement with these results. Possibly, these more global dynamics do not act on fine-grained stimulus-specific information but serve as a temporal reference frame, which in turn plays a crucial role in the attention and context-dependent organization of a functional network [12–18]. In addition, these methods require all the pairs of sites to be sufficiently strongly synchronized to extract phase delays from cross-correlograms. This may lead to a bias towards the responses and the electrodes that reflect strongest levels of β/γ synchronization, which, as evidence suggests, are associated with the smallest phase delays [12,13,15].

Our study could not exhaust all the possible stimuli and tasks, and the analysis was limited to the β/γ frequency range. In addition, the LFP electrodes spanned only distances below 1 mm. Hence, we cannot exclude the possibility that other experimental approaches and variables, presently not investigated, lead to larger delays in the LFPs and the more global signals.

Relative delays between the preferred discharge times and the phase of ongoing population oscillations, which are reflected in the LFP, have been described in the hippocampus and addressed as phase precession [11]. In this case, the relative firing times contain information about the animal's position in space. Moreover, the mechanism causing the precession has also been elucidated recently. The firing time delays were introduced because of systematic differences between the constant frequency of the population oscillation, which is synchronous across large groups of cells, and the frequency of intrinsic oscillations of individual cells, which is modulated by the strength of excitatory drive. It is likely that we deal with a similar phenomenon here, albeit in the β/γ frequency range, LFP reflects the global rhythm, to which a large population is synchronized, but individual cells of this population have distinct preferred firing times in the cycle, depending on stimulus features or top-down influence.

Fig. 3



Signal types differ in sensitivity to changes in stimulation condition. (a) The relative activation times of a set of LFP and MUA signals for two different stimulus conditions (illustrated on the right). The same colors indicate the same nodes (i.e., electrodes). (b) Black bars: sensitivity to stimulus conditions, κ, for all the five signal types. Gray bars: κ' (corrected) for the variability between split-half estimates. NS, non-significant; #marginally significant value, $P=0.077$; *$P<0.05$.

Conclusion

When synchronization in the β/γ range is strong, individual neurons fire action potentials at preferred times relative to the ongoing oscillation. Neuronal groups within larger structures such as a cortical column or even an entire cortical area may shift their phase collectively, which can give rise to phase shifts in the LFP and EEG signals, but these collective changes seem to be related to the organization of larger functional networks rather than the encoding of fine-grained stimulus-related properties. Accordingly, the information contained in the relative activation times of local clusters of neurons can only be retrieved by determining neuronal activity at the level of individual neurons.

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