EJN European Journal of Neuroscience FENS Federation of European Neuroscience Societies

Temporal coupling of field potentials and action potentials in the neocortex

Brendon O. Watson, Mingxin Ding & György Buzsáki

Review timeline:

Submission date:	14 August 2017
Editorial Decision:	09 October 2017
Revision received:	27 October 2017
Accepted:	23 November 2017

Editor: John Foxe

1st Editorial Decision 09 October 2017

Dear Dr. Watson,

Your manuscript was reviewed by two external reviewers and the editors. The reviews collectively indicate that your work has generated new and important information. However, there are several issues, mostly of the minor variety, that need to be clarified before we can proceed further with your manuscript for publication in EJN (see appended reviews). Also, please attend to the following list of issues in your revisions:

Corresponding author info & keywords need to be included on the title page
Some acronyms are not defined at first use
Ethical approval needs to be stated.
References need to be checked for missing information
Author contributions, COI and a data sharing statement are required
Figures need to be clearly labelled so readers know which is which
Abbreviation list needed

If you are able to respond fully to the points raised, we would be pleased to receive a revision of your paper within 30 days.

Thank you for submitting your work to EJN.

Kind regards,
John Foxe & Paul Bolam
co-Editors in Chief, EJN

Reviews:

Reviewer: 1 (Ian Fiebelkorn, Princeton University, USA)

Comments to the Author
The present manuscript, titled “Temporal coupling of field potentials and action potentials in the neocortex,” explores the relationship between spiking activity and the local field potential (LFP) using data from rat frontal cortex. This is a complex topic, certainly worthy of further investigation. As with previously published research, the present manuscript describes a strong correlation between spiking activity and high-gamma power (50-180 Hz), suggesting that power in this frequency range serves as a reasonable proxy for spiking activity. The manuscript then further explores whether this correlation differs by brain state (REM, non-REM, wake) and neuronal subtype (excitatory, inhibitory). The manuscript also reports correlations between spiking activity and lower-frequency LFP power, in some cases anti-correlations.

The manuscript definitely has the feel of a secondary analysis, meaning it feels a bit atheoretical. The introduction, for example, offers no hypotheses about how or why correlations between spiking activity and the LFP might differ by brain state or neuronal subtype. However, the manuscript does add considerable information to the existing literature. For example, it shows that the correlation between spiking activity and high-gamma power is highly robust at the level of neural populations, persisting across very different brain

states and across neuronal subtypes. The manuscript also includes a number of useful follow-up analyses.

See below for my specific comments.

(1) There are a number of typos and phrasing issues in the abstract and intro. These sections should be more carefully edited.
(2) The beginning of the "RESULTS" section is largely redundant with what was just stated in the very brief "METHODS" section.
(3) The x-axes in Fig. 1A need labels.
(4) Why are there so many fewer points plotted on the REM panel of Fig. 1C?
(5) I'm having a hard time interpreting the y-axis in Fig. 2A. Does this represent the correlation coefficient for each individual neuron, averaged across all neurons (within that subtype)? If so, these averages seem low, particularly for pE neurons. Are the results statistically significant?
(6) The authors compare the frequencies with the strongest spike rate to LFP power correlations (Fig. 2A) to the general frequency spectra (Fig. 2B), noting that these plots are dissimilar. In the text it suggests that these two measures did not directly correlate, which implies some sort of analysis was done. If all the authors are doing is making a naked eye, visual comparison, the text should better reflect that.
(7) In the section "LFP phase modulation of spikes," the authors write "We found that both cortical pE units and pI showed phase modulation at many frequency bands." However, studying the corresponding figure (Fig. 5C), it seems that their statistically significant results are limited to high-gamma for pI neurons for "wake" and delta for both neuronal subtypes for "NREM."

Reviewer: 2 (Kevin Whittingstall, Universite de Sherbrooke, Canada)

Comments to the Author
This work represents a thorough analysis of the relationship between the measured output (spikes) and LFPs in rat neocortex. Most importantly, the results are presented across cell types, frequency bands and brain states. The manuscript is well-written, methods are solid (though statistics are lacking in some areas) and the figures are of excellent quality. The final product is a detailed account of the underlying mechanisms giving rise to LFPs, which I am sure, will be referred to by many researchers conducting their own studies. I have a few comments aimed primarily at clarifying certain parts of the paper.

Methods
– Much of the results depend on the firing rate estimated from extracellularly recorded voltages. Yet, little to no detail is given regarding how exactly spikes were extracted from the broadband signal. What kind of amplitude and timing criteria were used to define spikes?
-How exactly were pE and pI defined, and more importantly, is this approach justified?

Results
-Figure 1C- the red and green lines are clear, but what does ~10 Hz spike rate (peak of blue curve) indicate?
-figure 1C, the low LFP-spike rare correlations for frequencies below ~20 Hz seems to be at odds with other studies (who report higher correlations). The authors may want to comment on this.
-Figure 5 clearly shows results when 'shuffling' the data. This is a good approach as it gives the reader a feel for the practical significance of the results. Please add this to Figure 2A.
-Page 9-how is distance from electrode computed?
-Page 9 - the statement associated with Figure 3C: 'It is visually evident that the highest frequency Bands are increasingly impacted by the largest amplitude spikes' is not clear to me. Where is this result? Same for figure 3d…is this result statistically significant?

-figures 5 and 7 are impressive

Discussion

-it is stated: 'Finally, we provide a new metric for quantifying the relationship between spiking and LFP, and show that it provides stronger correlations values than previous methods.' What are the authors referring to exactly here?

-it is stated: 'spatially resolved gamma power in identified dendritic regions can provide important information about the magnitude of upstream drive (Colgin et al., 2009; Berenyi et al., 2014; Schomburg et al., 2014;Fernandez-Ruiz et al., 2017).' The authors may want to include the results from Linden at al (https://www.ncbi.nlm.nih.gov/pubmed/22153380?access_num=22153380&link_type=MED&dopt=Abstract) and Butler et al., (https://www.ncbi.nlm.nih.gov/pubmed/28455370) who show that input correlations are equally important when interpreting LFP.

-the findings from Waldert et al. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936368/) should be

included when discussing movement-related EMG contributions.

Authors' Response 27 October 2017

We thank the reviewers for their constructive comments; they helped improve our manuscript. We will reply to each comment below inline.

Reviews:
Reviewer: 1
Comments to the Author
The present manuscript, titled "Temporal coupling of field potentials and action potentials in the neocortex," explores the relationship between spiking activity and the local field potential (LFP) using data from rat frontal cortex. This is a complex topic, certainly worthy of further investigation. As with previously published research, the present manuscript describes a strong correlation between spiking activity and high-gamma power (50-180 Hz), suggesting that power in this frequency range serves as a reasonable proxy for spiking activity. The manuscript then further explores whether this correlation differs by brain state (REM, non-REM, wake) and neuronal subtype (excitatory, inhibitory). The manuscript also reports correlations between spiking activity and lower-frequency LFP power, in some cases anti-correlations.

The manuscript definitely has the feel of a secondary analysis, meaning it feels a bit atheoretical. The introduction, for example, offers no hypotheses about how or why correlations between spiking activity and the LFP might differ by brain state or neuronal subtype. However, the manuscript does add considerable information to the existing literature. For example, it shows that the correlation between spiking activity and high-gamma power is highly robust at the level of neural populations, persisting across very different brain states and across neuronal subtypes. The manuscript also includes a number of useful follow-up analyses.

See below for my specific comments.

(1) There are a number of typos and phrasing issues in the abstract and intro. These sections should be more carefully edited.
Thank you, we have now addressed them.

(2) The beginning of the "RESULTS" section is largely redundant with what was just stated in the very brief "METHODS" section.
We have truncated some of the most repetitive language. We did allow for some degree of overlap of content given that many readers do not look at the Methods section

(3) The x-axes in Fig. 1A need labels.
Thank you, labeled now (seconds)

(4) Why are there so many fewer points plotted on the REM panel of Fig. 1C?
This is simply because the brain spends less time in REM sleep than in wake or nonREM so there are fewer time bins to show data for. Less time in REM sleep is universal across animals.

(5) I'm having a hard time interpreting the y-axis in Fig. 2A. Does this represent the correlation coefficient for each individual neuron, averaged across all neurons (within that subtype)? If so, these averages seem low, particularly for pE neurons. Are the results statistically significant?
The reviewer is correct, we show in 2A the mean per-cell correlation of firing rate against each frequency band. We have now added to the y-axis label to indicate this more clearly. The value of these averages is in good correspondence with the fact that individual cells are highly variable in their correlations, as show in figure 2B and something which we point out in text as well. The individual neuron is different from the population and we view that as an important finding.

We have also now added to the figure the results of shuffling spike rate data and frequency data across bins and the correlations are greater than chance at nearly all bands. See the partially-transparent shuffled data spanning zero in Figure 2A, these lines denote the 95% confidence intervals after shuffling spike time vs frequency band power.

(6) The authors compare the frequencies with the strongest spike rate to LFP power correlations (Fig. 2A) to the general frequency spectra (Fig. 2B), noting that these plots are dissimilar. In the text it suggests

that these two measures did not directly correlate, which implies some sort of analysis was done. If all the authors are doing is making a naked eye, visual comparison, the text should better reflect that.
The reviewer is correct that the comparison was not actually quantitative and was instead an illustration. We have now changed the text to reflect this.

(7) In the section "LFP phase modulation of spikes," the authors write "We found that both cortical pE units and pI showed phase modulation at many frequency bands." However, studying the corresponding figure (Fig. 5C), it seems that their statistically significant results are limited to high-gamma for pI neurons for "wake" and delta for both neuronal subtypes for "NREM."
We used Wilcoxon signed rank test to compare the mean resultant lengths (MRL) of real data and the mean of shuffled MRLs. The comparison is paired, right-tailed and the significance level was set as 0.01 after Bonferroni correction. The differences were found significant for all frequency bands of both pE and pI cells. It is true that the standard deviation of real and shuffled data was overlapping in some of the bands, but overall the real MRL was about 0.5 order of magnitude larger than the shuffled data even at the frequencies where they were the closest. And the paired, one-sided test could result in more tolerance with smaller group difference as shown in figure 5C.

Reviewer: 2

Comments to the Author
This work represents a thorough analysis of the relationship between the measured output (spikes) and LFPs in rat neocortex. Most importantly, the results are presented across cell types, frequency bands and brain states. The manuscript is well-written, methods are solid (though statistics are lacking in some areas) and the figures are of excellent quality. The final product is a detailed account of the underlying mechanisms giving rise to LFPs, which I am sure, will be referred to by many researchers conducting their own studies. I have a few comments aimed primarily at clarifying certain parts of the paper.

Methods
– Much of the results depend on the firing rate estimated from extracellularly recorded voltages. Yet, little to no detail is given regarding how exactly spikes were extracted from the broadband signal. What kind of amplitude and timing criteria were used to define spikes?
We now added these details. Broadband data was highpass filtered at 800Hz, then all signals more than 3.5 SD below the mean were considered candidate spikes. All candidate spikes were spike-sorted using klustakwik which employs PCA-based dimensionality reduction and Kmeans/EM clustering - further details of that algorithm are available in the paper cited in our manuscript: (Rossant 2016)

-How exactly were pE and pI defined, and more importantly, is this approach justified?
We now describe both the process and the justification in more detail in the methods section. We also refer the reader to a specific figure from an earlier publication using the same dataset to provide more clarity.

Results
-Figure 1C- the red and green lines are clear, but what does ~10 Hz spike rate (peak of blue curve) indicate?
The blue curve represents broadband gamma as it does in Fig 1A. To make this more clear, we have added a legend to this panel.

-figure 1C, the low LFP-spike rare correlations for frequencies below ~20 Hz seems to be at odds with other studies (who report higher correlations). The authors may want to comment on this.
We think the reviewer means to refer to Figure 2C, not 1C and we will comment based on that understanding.
We think the reviewer is right that this is a valuable element of our findings. We have now added both mention of this fact and pointers to both our data and that of others in the results section. In addition, we keep the section of our discussion devoted to this topic (see the paragraph starting with "As brain state varies,…").
To go into detail here: In Mukamel et al 2005, spike correlates are higher, but those are

population sums and so they represent general tendencies and do not show individual cell-to-cell variation in correlation. Nir et al 2007 show a range of individual spike-rate correlations with gamma power (see their figure 2A). Furthermore, based on their figure 1, we see a variety of brain states wherein gamma is coupled to spiking to greatly varying degrees. Specifically, their figure 1A shows a state with pauses of spiking and gamma power for ~500ms at a time. We think this represents a delta-oscillatory state which may be akin to nonREM sleep, while their lower figure appears to be a more aroused brain state with less fluctuating overall network activity. It is not clear in which precise state each analysis was done in that early work and it is specifically for this reason that we separate our analysis by brain state. In particular the large and well known co-modulation of both spike rate and gamma oscillation during UP and DOWN states in nonREM sleep can greatly elevate correlations between spike rate and gamma power and our Supplemental Figure 1 bears out this notion – see the fact that nonREM sleep changes depending on whether the bin size is longer or shorter than the ~1Hz delta fluctuation. We now discuss this to some extent in the text.

-Figure 5 clearly shows results when 'shuffling' the data. This is a good approach as it gives the reader a feel for the practical significance of the results. Please add this to Figure 2A.
Thank you for this suggestion, we have now added this information to Figure 2A.

-Page 9-how is distance from electrode computed?
We used Euclidean distance between shanks. Each shank of our electrodes is separated by 200µm. In some analyses we simply used signals from electrodes on neighboring shanks, i.e., 200µm away from the reference site. We have clarified this in the text.

-Page 9 - the statement associated with Figure 3C: 'It is visually evident that the highest frequency Bands are increasingly impacted by the largest amplitude spikes' is not clear to me. Where is this result? Same for figure 3d…is this result statistically significant?
We made brief mention of this in the original submission and have now fleshed out the discussion of significance in this section. We hope this improves reader comfort with the finding

-figures 5 and 7 are impressive
Thank you for this comment.

Discussion

-it is stated: 'Finally, we provide a new metric for quantifying the relationship between spiking and LFP, and show that it provides stronger correlations values than previous methods.' What are the authors referring to exactly here?
We are referring to the work shown in Figure 8 demonstrating that the correlation spectrum better predicts spike rates than other metrics used here, including better than those used by other researchers in the past. We have added a clarifying phrase now, hopefully that will leave less ambiguity.

-it is stated: 'spatially resolved gamma power in identified dendritic regions can provide important information about the magnitude of upstream drive (Colgin et al., 2009; Berenyi et al., 2014; Schomburg et al., 2014;Fernandez-Ruiz et al., 2017).' The authors may want to include the results from Linden at al (https://www.ncbi.nlm.nih.gov/pubmed/22153380?access_num=22153380&link_type=MED&dopt=Abstract) and Butler et al., (https://www.ncbi.nlm.nih.gov/pubmed/28455370) who show that input correlations are equally important when interpreting LFP.

-the findings from Waldert et al. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936368/) should be included when discussing movement-related EMG contributions.

We have now included these references in the manuscript. The urls are also appreciated.