nature portfolio

Peer Review File

Functional architecture of intracellular oscillations in hippocampal dendrites

Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*. Mentions of prior referee reports have been redacted.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript by Liao et al uses voltage imaging to investigate intracellular oscillations in hippocampal neurons. Fast two-photon imaging of both dendritic and somatic membrane potential dynamics were recorded in single pyramidal cells during a spatial navigation task. By combining voltage imaging with simultaneous local field potential recording, the dynamics of sub- and supra-threshold membrane potential were reported throughout the dendritic arbor in vivo. The study finds that local event rates are modulated by locomotion in distinct dendritic regions and an advancing gradient of dendritic theta phase occurs along the basal-tuft axis. The study also reports a hyperpolarization of the dendritic arbor during sharp-wave ripples, and finds the spatial tuning of dendritic representations dynamically reorganizes following place field formation. Overall, the study concludes that the electrical signaling in dendrites maps onto the anatomy of the dendritic tree across behavior, oscillatory network, and functional cell states. Overall, the resubmitted manuscript has changed little from the previous submission to [**Redacted**]. The Authors addressed some of my concerns, but all points were not adequately addressed.

The text and citations were improved when referring to previous electrical recordings from dendrites (which just required a slight word change). However, the introduction still does not provide adequate background to the questions tested, as it focuses on the uniqueness of the recording technique which then makes interpreting the relevance of the findings difficult. Please expand the introduction to provide relevant background to the questions addressed.

Further, the Figures have not changed substantially – esp Figure 1d which needs to be expanded to enable readers to assess the overlay of voltage imaging and electrophysiology. The authors state ‘We then compared the model-predicted ASAP3 response to single action potentials recorded in vivo to detected DEs in our dataset, and found close qualitative agreement’. However, it is evident (Figure 1d and S3) that the model-predicted ASAP3 action potential is not in ‘close qualitive agreement’ with the ‘single action potentials recorded in vivo’ as it is considerably wider. Please describe the signal to better reflect the data. Also, a more comprehensive characterization of the dendritic events as ‘fast’ and ‘slow’ is needed. Were the half-widths significantly different? Were the amplitude of the two different waveforms significantly different? An overlay to describe the DEs recorded would be useful.

Overall, the discussion is still lacking in drawing the relevance of the results together. For example, what is the relevance of DE amplitude being strongly modulated uniquely in the tuft dendrites? What is the relevance of phase progression of DE events in different dendritic regions? Is this a pathway specificity?

Since background levels of internal membrane-bound ASAP3 in the somatic compartment decreases the fluorescence changes recorded from the soma (as stated in reference to Figure 4), it is not viable to compare the fluorescence amplitudes in the different cellular regions which all have different surface-to-volume ratios (eg: tuft dendrites are considerably thinner than apical dendrites).

The authors state that they 'expect locally-generated events to be entrained to local theta phase (45°slope of the DE phase vs membrane potential phase relationship) and bAPs to occur preferentially at the peak of somatic theta (0°slope)'. However, it is also stated that 'unlike fast DEs, the distribution of slow events appear to be concentrated around 0° in all regions but the tuft'. This would suggest that the slow events are bAPs. However, this seems contradictory with other descriptions that state slow DEs are 'likely representing sodium spikes with a slower, NMDA receptor/voltage-gated calcium channel-mediated component' whereas fast DEs are reported as 'likely representing both locally generated and backpropagating action potentials in dendrites'. Please reconcile these statements.

Figure S9b - axis label is missing

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We believe that we have provided adequate background information in our Introduction aimed at balancing the technical novelty of our work and introducing the specific questions that it allows us to answer. Nevertheless, we have now significantly revised the Introduction to further highlight the goal of our study and the biological questions we sought to answer.

Further, the Figures have not changed substantially – esp Figure 1d which needs to be expanded to enable readers to assess the overlay of voltage imaging and electrophysiology. The authors state ‘We then compared the model-predicted ASAP3 response to single action potentials recorded in vivo to detected DEs in our dataset, and found close qualitative agreement’. However, it is evident (Figure 1d and S3) that the model-predicted ASAP3 action potential is not in ‘close qualitive agreement’ with the ‘single action potentials recorded in vivo’ as it is considerably wider. Please describe the signal to better reflect the data. Also, a more comprehensive characterization of the dendritic events as ‘fast’ and ‘slow’ is needed. Were the half-widths significantly different? Were the amplitude of the two different waveforms significantly different? An overlay to describe the DEs recorded would be useful.

We enlarged panel 1d on main Figure 1 as much as possible. We note that there is a very close correspondence between the Markov model response (purple) and the measured fluorescence response (green) in a voltage-clamped HEK293 cell at 37 °C and in vivo in response action potential waveform (black) from an in vivo recorded mouse dorsal hippocampal CA1 area pyramidal cell. We also note that we dedicate a whole supplementary figure (Fig. S3) for the Markov model, including higher magnification traces and we reference this supplementary figure in the main text together with Figure 1d. We now also added a reference to this supplementary Fiure to the legend of Figure 1d to guide the reader once more to this extended analysis.

Overall, the discussion is still lacking in drawing the relevance of the results together. For example, what is the relevance of DE amplitude being strongly modulated uniquely in the tuft dendrites? What is the relevance of phase progression of DE events in different dendritic regions? Is this a pathway specificity?

We note that due to space limitations, the Discussion cannot cover all the possible interesting and relevant points. Nevertheless, we have revised the Discussion to incorporate the specific points raised by the Reviewer above.

Since background levels of internal membrane-bound ASAP3 in the somatic compartment decreases the fluorescence changes recorded from the soma (as stated in reference to Figure 4), it is not viable to compare the fluorescence amplitudes in the different cellular regions which all have different surface-to-volume ratios (eg: tuft dendrites are considerably thinner than apical dendrites).

Thank you. Indeed, as requested for the previous round of reviews, we have primarily used relative (z-scored) measures of fluorescence signals across compartments in main figures and, in addition, we report absolute measures of these signals in supplementary figures (e.g. Fig. S6).

The authors state that they 'expect locally-generated events to be entrained to local theta phase (45°slope of the DE phase vs membrane potential phase relationship) and bAPs to occur preferentially at the peak of somatic theta (0°slope)'. However, it is also stated that 'unlike fast DEs, the distribution of slow events appear to be concentrated around 0° in all regions but the tuft'. This would suggest that the slow events are bAPs. However, this seems contradictory with other descriptions that state slow DEs are 'likely representing sodium spikes with a slower, NMDA receptor/voltage-gated calcium channel-mediated component' whereas fast DEs are reported as 'likely representing both locally generated and backpropagating action potentials in dendrites'. Please reconcile these statements.

We thank the Reviewer for this new comment. However, respectfully, it does not follow that simply because two events have the same theta preference (0° phase), they must be the same event. Other parameters, notably the much wider FWHM, strongly suggest these are *not* single bAPs, although they may correspond to the plateau component of somatic burst firing. Therefore, we state that our results are consistent with the interpretation above that DEs mainly correspond to individual sodium spikes, while slow events correspond to more global plateau-burst events, which comprise both fast sodium backpropagating action potential (burst) and slower voltage-gated calcium/NMDA-mediated (plateau) component. To clarify this further, we adjusted our definition of fast (DE) and slow events as such:

"DEs refer to fast sodium spike-like events, likely representing locally generated or backpropagating individual spikes in dendrites"

"Slow events "likely representing a combination of sodium spikes with a slower, NMDA receptor/voltage-gated calcium channel-mediated component (plateau-burst)".

Figure S9b - axis label is missing

Thank you. Fixed