The Journal of Physiology

Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro

Natalie Schieferstein, Ana Itzel Nuñez del Toro, Roberta Evangelista, Barbara Imbrosci, Aarti Swaminathan, Dietmar Schmitz, Nikolaus Maier, and Richard Kempter
DOI: 10.1113/JP285671

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The referees have opted to remain anonymous.

Review Timeline:

Submission Date:	12-Sep-2023
Editorial Decision:	30-Nov-2023
Revision Received:	24-Apr-2024
Editorial Decision:	11-Jun-2024
Revision Received:	19-Jul-2024
Accepted:	08-Aug-2024

Senior Editor: Katalin Toth

Reviewing Editor: Jean-Claude Béïque

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr Kempter,

Re: JP-RP-2023-285671 "Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro" by Natalie Schieferstein, Ana Itzel Nuñez del Toro, Roberta Evangelista, Barbara Imbrosci, Aarti Swaminathan, Dietmar Schmitz, Nikolaus Maier, and Richard Kempter

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Yours sincerely,

Katalin Toth
Senior Editor
The Journal of Physiology

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- 'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

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- The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the

Mean (SEM) alone is not permitted.

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EDITOR COMMENTS

Reviewing Editor:

This paper reports the results of electrophysiological recordings using both MEA/LFP and whole-cell recordings from mouse hippocampal slices to study features of sharp wave-ripple propagation in CA3 region of the hippocampus. This work has been reviewed by two expert reviewers who provided somewhat diverging account of the study. While they both recognize the overall quality of the work, they collectively raised a number of concerns that triggered varying levels of enthusiasm and novelty perception. Some significant technical concerns were raised that need to be satisfactorily addressed before publication. For instance, some of the propagation metric was seen by both reviewers as being somewhat obscure, and may bring unaccounted non-linearities that may bias velocity estimation (e.g., collapse of driving force during high frequency/amplitude bursts). Some alternative experimental approaches have been suggested to be better suited to answer the core set of questions being addressed here. The authors can expand on whether, or not, this is indeed the case.

REFEREE COMMENTS

Referee #1:

The paper entitled "Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro" by N. Schieferstein et al investigates the propagation of sharp wave ripples within CA3 in slice recordings. The authors use both MEA and whole-cell patch clamp recordings in combination with LFP to explore how SWRs propagate within CA3 circuits along the CA3 pyramidal cell layer. The paper is clearly written, with adequate statistical analysis of the data and appropriate conclusions. The combination of MEA and multiple single cell recordings are valid approaches to confirm the result that SWR travel from CA3b (possibly CA3c) to CA3a at the LFP and synaptic levels.

As discussed by the authors, divergent conclusions have been made with in vivo recordings in awake rodents. This is well discussed and possible explanations are provided, albeit it does indicate that the question of SWR propagation is tightly linked to the experimental conditions. Nevertheless, this study provides valuable information to the field.

Minor points:

- the rationale for the use of the slope in ms/% is understandable, it would like interesting to see the absolute values of the delay, and the distribution of absolute distances between CA3c and CA3a.

- In the last 'key point', line 11: the new insights brought by this paper on the dynamics of SWR in CA3 is certainly useful for models and understanding wave generation mechanisms, but it is not clear that this will provide understanding of their functional role at this point.

- Regarding Figure 2 and Slice ID 25, it appears that the mean regression slope may not accurately represent the mean of these distributions. However, it's worth noting that this discrepancy may be due to the distribution's weak bimodality.

- In the discussion, it is unclear how the propagation velocity obtained in line 454 because it is calculated for the cEPSCs and cIPSCs at 439 and 417. Please clarify the difference between the two.

- The research performed could become a lot further informative if CA1 SWR would also be recorded. The underlying question to confirm being: are the SWR propagating through CA3c to CA3a are more likely to trigger SWR in CA1 than the ones that don't propagate.

- The use of mice aged 3-9 weeks captures a critical developmental period spanning from early post-weaning juveniles to young adults. However, variations in physiology and behavior within this age range should be considered and could be commented in text.

- The authors identified a solid bibliography to contextualize their work. However, "Sharp-Wave Ripples Orchestrate the Induction of Synaptic Plasticity during Reactivation of Place Cell Firing Patterns in the Hippocampus" from Josef H.L.P. Sadowski, Matthew W. Jones, and Jack R. Mellor addresses the induction of LTP in CA3 and CA1 during SWR. Their work provides valuable insights into the role of SWRs in synaptic plasticity and memory consolidation. Citation of this paper would enhance the current study by providing relevant context and supporting the claims regarding the functionality of SWR.

- The last paragraph of the discussion highlights the interaction between excitatory and inhibitory neurons in the generation of sharp wave-ripples (SPWs) in CA3 and suggests that the directional preference of SPW propagation is likely influenced by differences in the circuit architecture within CA3. The authors support this claim mostly by computational models and simulations references. They conclude on the fact that the velocity in CA3 is well understood. The clarity of this claim could be improved.

***

Referee #2:

This is a carefully-executed study of the propagation of population activity through acute adult hippocampal slices. Overall, the exposition is clear. The authors investigated the propagation of sharp wave-ripple discharges in mouse hippocampal slices using multi-electrode arrays and single-cell recordings. They found a preferred propagation of sharp ways from CA3c toward CA3a. The major weaknesses of the study are related to the preparation and the recording techniques. Comments:

1. Preparation: acute hippocampal slices have certainly been a popular preparation for electrophysiology. However,

a. Isolated acute adult hippocampal slices do not exhibit spontaneous network activity. They need to be disinhibited in some way. For example by pharmacologically blocking GABAA receptors, or increasing extracellular potassium (PMID 14984409). Or perhaps in this case as a result of 18-20 mbar vacuum pressure applied during slice placement onto the "bed of nails" electrode array: trauma has well-documented disinhibitory effects (PMIDs 8753889; 22442068).

b. The trauma associated with hippocampal slicing (PMID 22442068) and the variation in the angle at which the hippocampal slices are prepared results in substantial variance in the neural network and net axonal vectors present from slice to slice. This paper is quite good at reporting slice-to-slice variance, but it begs the question: why not do this study in situ / in vivo?

2. Recording techniques:

a. microelectrode arrays are certainly useful, but more so in vivo. In vitro, there are much more informative means to study population activity, for example by cellular calcium imaging.

b. The intracellular records use positive and negative holding potentials and voltage clamp to obtain waveforms that can be used to measure the propagation of population activity. However, being measurable is not the same thing as being meaningful. The cellular events during population activity are a mixture of inhibitory and excitatory membrane conductances with a net reversal potential that varies during the event. Large voltage-dependent membrane conductances will contribute to this mixed conductance and to the time variance in reversal potential. The variance in reversal potential will contribute to the apparent propagation speed. It is not clear from the data presented that the intracellular records are a useful measure of propagation (line 454).

• Analysis: this can be considered a minor issue, but it is not clear why the authors choose such nonintuitive measures of propagation: often the inverse of conduction velocity, and often substituting the fraction of the CA3-CA1 distance for actual distance in mm. Because the electrode spacing is known, it should be easy to add conduction velocity in mm/sec to the various tables and figures. This would make the data much easier to compare to other studies of hippocampal conduction (PMID 12944517).

END OF COMMENTS

Confidential Review **12-Sep-2023**

EDITOR COMMENTS

Reviewing Editor:

This paper reports the results of electrophysiological recordings using both MEA/LFP and whole-cell recordings from mouse hippocampal slices to study features of sharp wave-ripple propagation in CA3 region of the hippocampus. This work has been reviewed by two expert reviewers who provided somewhat diverging account of the study. While they both recognize the overall quality of the work, they collectively raised a number of concerns that triggered varying levels of enthusiasm and novelty perception. Some significant technical concerns were raised that need to be satisfactorily addressed before publication. For instance, some of the propagation metric was seen by both reviewers as being somewhat obscure, and may bring unaccounted non-linearities that may bias velocity estimation (e.g., collapse of driving force during high frequency/amplitude bursts). Some alternative experimental approaches have been suggested to be better suited to answer the core set of questions being addressed here. The authors can expand on whether, or not, this is indeed the case.

We would like to extend our thanks to the Reviewing Editor for their recognition of the overall quality of our work and for supporting the revision of our manuscript. Additionally, we wish to express our sincere gratitude for the thoughtful and detailed reviews provided by the expert reviewers.

Below you will find our responses to the questions and issues raised by the reviewers, point by point. The critiques have indeed served as a trigger for significant improvements in our study. We firmly believe that our manuscript has been enhanced based on the reviewers' comments.

We look forward to any further suggestions and are fully prepared to make additional modifications, if necessary, to meet the publication standards of *The Journal of Physiology*.

Thank you once again for this opportunity to improve our manuscript,

Natalie Schieferstein,
Nikolaus Maier,
Richard Kempter,
(on behalf of all co-authors)

REFEREE COMMENTS

Referee #1:

The paper entitled "Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro" by N. Schieferstein et al investigates the propagation of sharp wave ripples within CA3 in slice recordings. The authors use both MEA and whole-cell patch clamp recordings in combination with LFP to explore how SWRs propagate within CA3 circuits along the CA3 pyramidal cell layer. The paper is clearly written, with adequate statistical analysis of the data and appropriate conclusions. The combination of MEA and multiple single cell recordings are valid approaches to confirm the result that SWR travel from CA3b (possibly CA3c) to CA3a at the LFP and synaptic levels.

As discussed by the authors, divergent conclusions have been made with in vivo recordings in awake rodents. This is well discussed and possible explanations are provided, albeit it does indicate that the question of SWR propagation is tightly linked to the experimental conditions. Nevertheless, this study provides valuable information to the field.

Response: We thank this referee for the generally positive assessment of our manuscript.

Minor points:
- the rationale for the use of the slope in ms/% is understandable, it would like interesting to see the absolute values of the delay, and the distribution of absolute distances between CA3c and CA3a.

Response: To indicate the distribution of absolute distances between CA3c and CA3a, we added in Fig. 2, Fig. 4*D*, and Fig. 5 spatial scale bars (similar to the 200 µm scale bar in the example in Fig. 1*D*2); furthermore, we now describe the distributions of lengths of CA3 (MEA and whole-cell data sets) in the text of the Results on Figs. 2 and 5. Note that absolute delays (or latencies) were already shown in most of our previous figures (Fig. 1*C*2, Fig. 1*D*2, Fig. 2 (right, now middle), Fig. 3*A*, Fig. 4*D*, Fig. 5 (right)).

- In the last 'key point', line 11: the new insights brought by this paper on the dynamics of SWR in CA3 is certainly useful for models and understanding wave generation mechanisms, but it is not clear that this will provide understanding of their functional role at this point.

Response: We agree with the reviewer. We have revised this key point and omitted the last clause "..., furthering our understanding of their potential functional role." The last key point now reads:
"These new insights into the dynamics of sharp waves in the CA3 network will inform future experiments and theoretical models of sharp wave generation mechanisms."

- Regarding Figure 2 and Slice ID 25, it appears that the mean regression slope may not accurately represent the mean of these distributions. However, it's worth noting that this discrepancy may be due to the distribution's weak bimodality.

Response: Indeed, in almost all slices the distribution of regression slopes is unimodal, and the mean regression slopes represent the distributions well. But in slice ID:25 in which the distribution of regression slopes is bimodal, the mean does not represent the distribution well. To better characterize this slice, we have added in the text the value of the regression slope at the large peak and mention that there is also a (weaker) peak with slope of opposite polarity, and all values are of similar order of magnitude. The updated text in the Results (line 331) now reads "weak bimodality only in slice ID:25 with peaks at about 0.10 ms/% and -0.05 ms/% and a mean regression slope of 0.050 ms/%".

- In the discussion, it is unclear how the propagation velocity obtained in line 454 because it is calculated for the cEPSCs and cIPSCs at 439 and 417. Please clarify the difference between the two.

Response: The propagation velocities mentioned in the Discussion (previous line 454) "(... cEPSCs: 0.14 m/s and cIPSCc: 0.10 m/s)" are a short version of the velocities reported in the Results (previous last paragraph, line 439): "0.14 [0.10, 0.19] m/s for excitation and 0.10 [0.07, 0.13] m/s for inhibition (median and 99% bootstrap CI, see Fig. S3)". In contrast, the values mentioned in previous line 417 of the Results ("3.06 ms/mm for excitation (Fig. 6*B*1) and 7.18 ms/mm for inhibition (Fig. 6*B*2)") are grand average *slopes.*

Note that our estimate of the average propagation velocity (previous lines 439/454) is not the "simple" inverse of the grand average slopes (previous line 417): Propagation slopes are signed, indicating the direction of propagation. Since average propagation speed should be independent of direction, it is crucial to take the *absolute* value of the propagation slopes *before* averaging and inverting. This is especially relevant in the whole-cell data set, in which several slices showed propagation slopes in both directions (Fig. 5, left). A simple inverse of the average *signed* slopes would thus *over*estimate the speed.

Since most slices in the MEA dataset exhibit slopes of largely the same sign, we had previously used the simple inverse of the grand average slope as a speed estimate on this data set. We realize, however, that the use of different speed estimates for the two data sets is potentially confusing. We have therefore adopted the "proper" speed estimate for the MEA data as well (i.e. inverse of average *absolute* propagation speed). We now explain early on in the Results for the MEA data: "...speeds were derived as the inverse of the absolute slope". Furthermore, we have extended Fig. 2 and Fig. 5 by an additional right panel showing the

distribution of propagation speeds for all slices, hoping that this illustration aids the understanding of the speed calculation.

- The research performed could become a lot further informative if CA1 SWR would also be recorded. The underlying question to confirm being: are the SWR propagating through CA3c to CA3a are more likely to trigger SWR in CA1 than the ones that don't propagate.

Response: We appreciate the suggestion for further experiments, which seem promising. In support of the hypothesis addressed by this reviewer, it is well established that SWR events in CA3 and CA1 are coupled, with CA3 events leading those in CA1s. This coupling has been demonstrated through various studies, both *in vitro* (e.g. Maier et al., 2002, 2003, 2009, PMCIDs: PMC2290340, PMC2343079, and PMC2732900; Both et al., 2008, PMID: 18493949; Imbrosci et al., 2021, PMCID: PMC9239734); and *in vivo* (e.g. Buzsaki, 1986, PMID: 3026567; Ciscsvari et al., 2000, PMID: 11144366; Oliva et al., 2016, PMCID: PMC8138857). Because of the specific wiring from subregions of CA3 to subregions of CA1 (Ishizuka et al., 1990, PMID: 2358523), propagation of SPW-Rs within CA3 could indeed be related to propagation of SPW-Rs within CA1. It would thus also be interesting and reasonable to know whether the propagation of SPW-Rs within CA3 is somehow related to triggering (and propagation) of SPW-Rs in CA1.

We think that a test of the hypothesis suggested by the reviewer is not easy. In terms of our main result (preferred *direction* of propagation), we would like to phrase a null hypothesis: propagation direction of SPW-Rs within CA3 does not affect their propagation to CA1. A test of this hypothesis is challenging because our results (MEA and whole-cell recordings) indicate that there is a strong preference of propagation direction from CA3c towards CA3a; the test would therefore rely on a small (and so far not significant) subsample of slices in which SPW-Rs propagate in the opposite direction. Thus, to achieve a significant result that depends on a small subsample, we likely would need a large dataset, possibly much larger than our current dataset.

Which methods could be used to obtain such a large new dataset? We cannot use the MEAs utilized in our study because the small area covered by the electrode array prohibits recording from CA3 and CA1 simultaneously. We also cannot use whole-cell recordings because of the short recording time for each cell (compare #events in Table 1 for MEA and Table 2 for whole-cell recordings). Instead we could rely on a setup in which a small number of separate LFP electrodes target both CA3 and CA1. In such a setup, results on propagation within CA3 (requires at least 2–3 electrodes) are expected to be much more variable than in

the MEA setup (many channels of interest as indicated by the dots in Fig. 1*C*2 and Fig. 2, right) (we think that a smaller number of electrodes (2–3) is also a major reason for the larger variability of whole-cell recordings compared to MEA recordings). Because of the specific wiring from subregions of CA3 to subregions of CA1, it would be advisable in new experiments to also use several electrodes in the different subregions of CA1 (at least 2-3 electrodes).

In summary, to test the mentioned hypothesis, we expect that we need more slices and longer recording times than in the MEA recordings. Moreover, we need simultaneous recording from many LFP electrodes. Or we would need to establish recordings using a much larger MEA that could cover CA3 and CA1 simultaneously. Together, this would be a considerable extension of our current study.

Because it is still not clear enough how large a dataset should be to be able to reject the null hypothesis, and because the propagation of SPW-Rs from CA3 to CA1 is already well established, we did not perform such new experiments. We feel that this would be a topic for a separate follow-up manuscript.

- The use of mice aged 3-9 weeks captures a critical developmental period spanning from early post-weaning juveniles to young adults. However, variations in physiology and behavior within this age range should be considered and could be commented in text.

Response: We agree that 3-9 weeks of age is a wide range. However, basic features of sharp wave ripple complexes are well established and stable from the third week of age onwards, which is supported by our own observations and by at least two further publications: Buhl and Buzsaki (2005) PMC: 1851000 wrote: “Although SPW is the first detectable postnatal network pattern in the hippocampus, associated ripples begin to emerge only at the end of the second postnatal week, growing to near adult-like ripple oscillations by P20. Once they emerge their frequency remain invariant across development.” Similarly, Wong et al. (2005) PMID: 15961234 investigated the postnatal development of sharp waves (which they referred to as SRFPs, spontaneous rhythmic field potentials) and concluded that “SRFP frequencies stabilized in the 1–3 Hz range after postnatal day 15 and were not correlated with ages in the period of postnatal days 15–28 [...]. Together [...], these data suggest that SRFPs develop in a discrete time window during the second postnatal week and then persist into adulthood.”

To better justify the chosen range of age of mice used in our experiments and to emphasize that the age range is not critical in the context of our current investigation, we have included a new analysis supporting this notion (new Figure A1), and we have updated the beginning of “Materials and Methods - Subjects”, which now reads:

“A total of 29 male C57BL/6N mice, aged 3 to 9 weeks, were analyzed. Given that basic features of SPW-Rs, including the occurrence of events and ripple frequency, are stable from the third week of age onwards (Buhl and Buzsáki, 2005; Wong et al., 2005), the broad range of ages chosen for our analysis is justified. In a subset of experiments, we tested for possible correlations between propagation speed, directionality, and age. However, neither parameter showed a significant correlation, corroborating our assumption that was based on the published results (Fig. A1).”

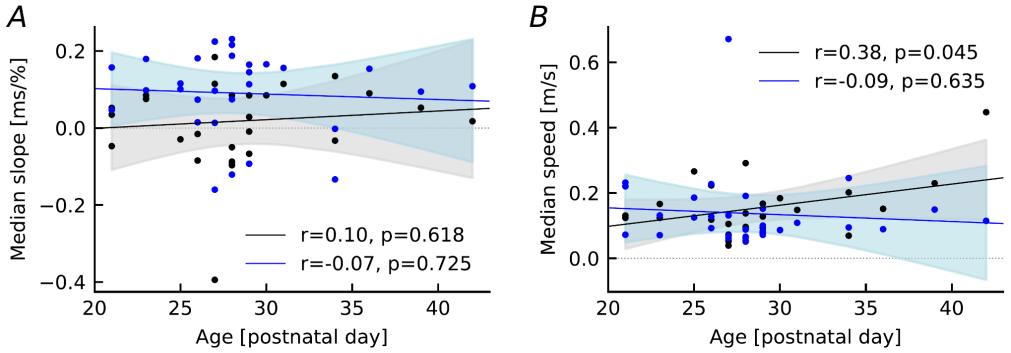

Figure A1 (new): No significant correlation between SPW propagation and mouse age in whole-cell experiments. A, Median propagation slope [ms/%] (Fig. 5, left; Fig. 6A) versus mouse age. B, Median propagation speed [m/s] (Fig. 5, right; Fig. 6E) versus mouse age. Black: derived from excitatory cPSCs, blue: derived from inhibitory cPSCs. Solid lines and shaded areas: linear regressions and confidence intervals. Legend indicates Pearson correlation coefficient and p-value.

- The authors identified a solid bibliography to contextualize their work. However, "Sharp-Wave Ripples Orchestrate the Induction of Synaptic Plasticity during Reactivation of Place Cell Firing Patterns in the Hippocampus" from Josef H.L.P. Sadowski, Matthew W. Jones, and Jack R. Mellor addresses the induction of LTP in CA3 and CA1 during SWR. Their work provides valuable insights into the role of SWRs in synaptic plasticity and memory consolidation. Citation of this paper would enhance the current study by providing relevant context and supporting the claims regarding the functionality of SWR.

Response: Thank you for this suggestion! We agree and have now cited this reference, Sadowski et al. (2016), in the first paragraph of the Introduction: “During SPW-R events, hippocampal ensembles are reactivated (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Nadasdy et al., 1999; Lee and Wilson, 2002). Furthermore, sharp wave-associated ripples, and in particular the firing of neurons reactivated during SPW-Rs, have been demonstrated to contribute to synaptic plasticity (King et al., 1999, Sadowski et al., 2016).”

- The last paragraph of the discussion highlights the interaction between excitatory and inhibitory neurons in the generation of sharp wave-ripples (SPWs) in CA3 and suggests that the directional preference of SPW propagation is likely influenced by differences in the circuit architecture within CA3. The authors support this claim mostly by computational models and simulations references. They conclude on the fact that the velocity in CA3 is well understood. The clarity of this claim could be improved.

Response: Many thanks for pointing out the overly strong claim in our Discussion. In response to your comment, we have now revised the last sentence of this section: We have removed the phrase "Together, the speed of propagation of activity within CA3 microcircuits seems to be quite well characterized, although…" and now conclude with "However, a comprehensive understanding of the mechanisms that trigger SPW-Rs and mediate their propagation in CA3 microcircuits remains an area for future investigation."

Referee #2:

This is a carefully-executed study of the propagation of population activity through acute adult hippocampal slices. Overall, the exposition is clear. The authors investigated the propagation of sharp wave-ripple discharges in mouse hippocampal slices using multi-electrode arrays and single-cell recordings. They found a preferred propagation of sharp ways from CA3c toward CA3a. The major weaknesses of the study are related to the preparation and the recording techniques.

Comments:
1. Preparation: acute hippocampal slices have certainly been a popular preparation for electrophysiology. However,

a. Isolated acute adult hippocampal slices do not exhibit spontaneous network activity. They need to be disinhibited in some way. For example by pharmacologically blocking GABAA receptors, or increasing extracellular potassium (PMID 14984409). Or perhaps in this case as a result of 18-20 mbar vacuum pressure applied during slice placement onto the "bed of nails" electrode array: trauma has well-documented disinhibitory effects (PMIDs 8753889; 22442068).

Response: Thank you for highlighting these important considerations. In our *in vitro* approach to studying sharp wave/ripples (SPW-Rs) utilizing mouse brain slices, we do not require the use of pharmacological agents or external electrical stimulation. This technique, established since the early 2000s, has been demonstrated by the foundational work of Papatheodoropoulos & Kostopoulos (*Brain Res Bull*, 2002; PMID: 11849825), Maier et al. (*J Physiol*, 2002, 2003; PMIDs 12042356 and 12807984), Kubota et al., 2003 (*J Neurophysiol,* PMID 12522161), and Wu et al. (*J Neurophysiol,* 2005; PMID 15772241). Notably, our slice model does not depend on $GABA_A$ receptor blockers or elevated extracellular K^+ for the generation of SPW-Rs. This model has facilitated significant mechanistic insights into SPW-Rs, as demonstrated by research from internationally renowned laboratories led by Draguhn, Gloveli, Gulyás, Hájos, Heinemann, Humeau, Ikegaya, Menendez de la Prida, Patrylo, Papatheodoropoulos, Stork, and Vicini. Their collective work has greatly expanded our understanding of the dynamics and functional significance of SPW-Rs. Regarding recording environments, spontaneously occurring SPW-Rs *in vitro* have been explored using various recording systems, including interface chambers (which maintain a thin film of fluid over the tissue), submersion chambers (where the tissue is fully submerged in ACSF), and multielectrode array (MEA) techniques. The MEA system employed in our study utilizes planar arrays of electrodes as opposed to “bed of nails” arrays. This choice reflects our aim to achieve detailed and minimally perturbed recordings of neural activity.

In the context of activity induction in hippocampal slices addressed by this reviewer, Karlócai et al. (*Brain*, 2014; PMID: 24390441) directly compared the cellular and network properties of spontaneously occurring sharp wave-ripple (SPW-R) events – with no need for stimulation – to those of pharmacologically induced network activities. These included activities evoked by high potassium, 4-aminopyridine, ACSF with nominally zero magnesium, and gabazine. They identified distinct differences between the spontaneous SPW-R events and the induced network patterns in amplitude, duration, and the instantaneous rate of multi-unit firing. Moreover, they observed neuron type-specific differences in spiking characteristics. Notably, pyramidal neurons and fast-spiking basket interneurons showed increased firing rates during pharmacological activity compared to spontaneous SPW-Rs. Interestingly, fast-spiking basket interneurons entered a depolarization block at the peak of network activity under pharmacological stimulation, tilting the excitation-to-inhibition balance towards excitation during this period.
In summary, spontaneous SPW-Rs exhibit distinctly different basic network and cellular properties compared to pharmacologically evoked patterns.

In direct response to the reviewer's point, we have added an informative paragraph in the Methods section to offer a more comprehensive overview of the *in vitro* model of this activity pattern. This paragraph ("*In vitro* model of SPW-Rs") now reads:

"In this experimental system, SPW-Rs arise spontaneously in slices bathed in standard ACSF. This implies that neither pharmacological nor electrical stimulation is required for their induction (Maier et al., 2002; Maier et al., 2003; Papatheodoropoulos & Kostopoulos, 2002; Kubota et al., 2003; Hájos et al., 2013; Kanak et al., 2013; Chiovini et al., 2014; Moradi-Chameh et al., 2014; Zarnadze et al., 2016; Jiang et al., 2018; Norimoto et al., 2018; Çalışkan et al., 2023; El Oussini et al., 2023). It should be noted that patterns of network activity can also be *induced* in hippocampal slices, e.g., following blockade of $GABA_A$ receptors or increases in extracellular K^+ or decreases in Mg^{2+}; however, these patterns are thought to have characteristics of pathological network activity and therefore serve as models for epileptiform or interictal events (see, e.g., Schneiderman, 1986; Wong and Traub, 1983; Khazipov et al., 2004; Karlócai et al., 2014)."

b. The trauma associated with hippocampal slicing (PMID 22442068) and the variation in the angle at which the hippocampal slices are prepared results in substantial variance in the neural network and net axonal vectors present from slice to slice. This paper is quite good at reporting slice-to-slice variance, but it begs the question: why not do this study in situ / in vivo?

Response: We agree with the reviewer that the trauma inherent to the slicing procedure is a crucial factor that should not be underestimated. This concern is highlighted in previous research from the lab of Kristin Harris, notably in the study by Kirov et al. (*Neuroscience* 2004; PMID: 15219670), which demonstrated that dendritic spines tend to disappear immediately after slicing, particularly when slicing is done in ice-cold ACSF. The spines were observed to re-emerge after more than 30 minutes. This finding underlines the sensitivity of neuronal structures to the slicing process. In a similar vein, we observe that after the initial slicing-induced synaptic 'silence', sharp wave/ripples (SPW-Rs) in slices gradually build up following the preparation, reaching a plateau at approximately 1.5 to 2 hours.

We further concur with the reviewer that, ultimately, *in vivo* experiments are ideal for directly investigating the propagation of SPW-Rs within the CA3 subfield, and we acknowledge the unmatched physiological relevance of such studies. However, our focus was to study both local field potentials (LFPs) and intracellular (subthreshold, synaptic) signaling in the context of SPW propagation in CA3, which required simultaneous recordings from at least two neurons. While in principle, simultaneous whole-cell recording from various neurons *in vivo* is technically feasible, it is extremely challenging in practice, especially in a deep brain structure like the hippocampal CA3 area, where issues such as electrical access and mechanical stability become pronounced. We therefore believe that the *in vitro* approach we have chosen is justified and ideally suited as it helps to overcome technical challenges and limitations of patch-clamp recording *in vivo*. Our approach provides comprehensive insights into neuronal activity during SPW-R propagation, offering a balance between experimental feasibility and the depth of understanding of these complex phenomena.

2. Recording techniques:

a. microelectrode arrays are certainly useful, but more so in vivo. In vitro, there are much more informative means to study population activity, for example by cellular calcium imaging.

Response: While cellular calcium imaging indeed offers the ability to capture activity from numerous cells simultaneously in slices, it is crucial to delve into specific considerations within the framework of our research, focusing on the propagation of SPW-R activity:

1) **Temporal resolution**: In our context, a key disadvantage of calcium imaging is its relatively low temporal resolution, which is typically around 5 ms even when deconvolution is applied (Yang Zhang et al., *Nature* 2023; PMCID: PMC10060165). This is in stark contrast to the much higher temporal resolution (< 0.1 ms) that can be achieved with electrophysiological approaches such as multi-electrode arrays (MEAs), which measure voltage directly. Since sub-millisecond precision is required to assess SPW-R propagation,

multi-electrode recordings are essential. Please note that our MEA dataset yielded SPW-R propagation latencies in the range of +/- 5 ms at most (see Fig. 3*A*).

2) **Definition and composition of LFP activity**: Sharp wave/ripple (SPW-R) activity is characterized by a local field potential (LFP) signature that is composed of both subthreshold and suprathreshold neural network activity. The measurement of SPW-R activity therefore requires recording techniques that can resolve local voltage gradients. While previous studies have integrated LFP recordings with cellular Ca^{2+} imaging, it is critical to recognize (**a**) that the cellular ("ensemble") activity associated with SPW-Rs is distributed across the observed neuronal network and (**b**) that it exhibits event-to-event variability in terms of the recruitment of active neurons. Consequently, cell calcium imaging proves insufficient for accurately characterizing *properties of LFP signatures*, such as propagation.

In summary, cellular Ca^{2+} imaging is invaluable for recording spike activity in distributed active networks, such as neuronal ensembles during SPW-Rs. However, given the mentioned constraints regarding LFP generation and temporal resolution, Ca^{2+} imaging is unsuitable for addressing propagation of SPW-Rs. Therefore, we affirm that our approach, which complements multi-electrode array and microelectrode whole-cell recordings, was well suited to accomplish our objectives.

b. The intracellular records use positive and negative holding potentials and voltage clamp to obtain waveforms that can be used to measure the propagation of population activity. However, being measurable is not the same thing as being meaningful. The cellular events during population activity are a mixture of inhibitory and excitatory membrane conductances with a net reversal potential that varies during the event. Large voltage-dependent membrane conductances will contribute to this mixed conductance and to the time variance in reversal potential. The variance in reversal potential will contribute to the apparent propagation speed. It is not clear from the data presented that the intracellular records are a useful measure of propagation (line 454).

Response: We appreciate this reviewer's critical perspective concerning the interpretation of voltage-clamp recording of synaptic activity during sharp wave/ripple population events. In particular, this reviewer addresses (**a**) the temporal overlay of inhibitory and excitatory membrane conductances and the resulting time-dependent changes of reversal potential; and (**b**) a potential influence of these voltage-dependent membrane conductances on population propagation inferred from voltage-clamp experiments.

Concerning (**a**), voltage-clamp experiments are an established method to counter the complexity of mixed synaptic currents. In detail, recording at a holding potential of -60 mV,

that is close to the equilibrium potential of Cl^- (and hence minimizes $GABA_A$ receptor mediated synaptic inputs/conductances – 'inhibition') or, additionally, +6 mV, that is close to the equilibrium potential of Na^+ (and hence minimizes AMPA receptor mediated synaptic inputs/conductances – 'excitation'), is a suitable and established way to address excitatory or inhibitory synaptic conductances. Due to minimal driving forces at the given reversal potential, the respective other component is quasi-isolated and thus accessible to investigation. Within the range of inherent limitations – e.g., space clamp constraints – this approach is valid to address excitatory and inhibitory synaptic conductances underlying neuronal population events and has been previously applied in studies analyzing the propagation of neuronal network activity (e.g., Pastoll et al.: *Neuron* 2013, PMID: 23312522; Swaminathan et al.: *Cell Reports* 2018, PMID: 29847786; Rozov et al.: *J Neurosci* 2020, PMCID: PMC7605420; Imbrosci et. al: *Cell Reports* 2021, PMCID: PMC9239734).

In response to the referee's point (**b**), we wish to highlight the following two aspects: (1) The focus of this part of our analysis is to compare the spread of inhibitory and excitatory synaptic network activity during sharp-wave/ripple (SPW-R) events. This analysis is based on the ***onset times*** of the composite excitatory and inhibitory synaptic currents as a proxy to quantify their propagation, as illustrated in Fig. 4*D*. It is important to note that voltage-dependent changes in reversal potential, which may occur during the span of a SPW-R event lasting several tens of milliseconds, do not influence our chosen readout parameter. This selection was made because the onset times provide a reliable measure of the very initial phase of synaptic activity, but are unaffected by subsequent voltage-dependent changes. Therefore, we assert that our analysis method is both meaningful and appropriate. (2) Our methodology employed an intracellular solution containing Cs^+ and QX-314, chosen for their effectiveness in blocking K^+- and fast Na^+ channels. This selection substantially reduces significant K^+-based conductances and Na^+-driven spiking activities, as documented in Monier et al. (*J Neurosci Methods*, 2008; PMID: 18215425) or in Dallas/Bell [eds.], 2021 (springer.com/book/10.1007/978-1-0716-0818-0).

Therefore, we are convinced that together, both the selection of the onset time as the key variable and the application of intracellular channel inhibitors counter potential distortions from membrane conductances in our estimation of synaptic activity propagation during SPW-R events.

• *Analysis: this can be considered a minor issue, but it is not clear why the authors choose such nonintuitive measures of propagation: often the inverse of conduction velocity, and often substituting the fraction of the CA3-CA1 distance for actual distance in mm. Because the electrode spacing is known, it should be easy to add*

conduction velocity in mm/sec to the various tables and figures. This would make the data much easier to compare to other studies of hippocampal conduction (PMID 12944517).

Response: Many thanks for bringing up these points. Let us first motivate in detail the "slope" and the "%" measures before we summarize the changes we made in response to this comment.

First, we start by reporting propagation "slope" (and not its inverse, the "velocity"), as it is the immediate result of our linear regression analysis (SPW arrival latencies vs channel position). The distribution of slopes was almost always unimodal (Fig. 2 and Fig. 5, *left*), and therefore the mean (or the median) describe the distribution well. Using the "velocity" instead leads to histograms which are harder to quantify, with many outliers at large positive values (corresponding to slopes close to 0, see previous Fig. S2, now revised Fig. 5, *right*).

Second, we used "percent" (and not the absolute length of CA3 str. pyr. in millimeters) for better comparison across slices with CA3 of various sizes (Results, previous line 316*f*). This enabled us to visualize propagation across CA3 for all slices in one graph (Fig. 2 and Fig. 5, previously *right*, now *middle* panel). We did, however, also report slopes in absolute units of s/m (see previous Fig. 3*C*).

In addressing this comment, we have expanded our Figures to allocate equal space for reporting both slopes and speeds, incorporating relative (%) and absolute (mm) spatial units.

(**i**) In Figs. 2 (*middle*), Fig. 4*D*, and Fig. 5 (*middle*), spatial scale bars have been incorporated, akin to the 200 μm scale bar depicted in Fig. 1*D*2.

(**ii**) A new right panel has been introduced to Figs. 2 and 5, illustrating the distribution of propagation speeds, with m/s utilized as the most intuitive unit of measurement.

(**iii**) The summary Figs. 3 and 6 have been expanded to include both slopes and speeds in both relative (ms/% or % /ms) and absolute units (s/m or m/s).

We believe that these adjustments effectively address the concerns raised by the reviewer and improve the clarity of our analysis for the readers.

Dear Dr Kempter,

Re: JP-RP-2024-285671R1 "Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro" by Natalie Schieferstein, Ana Itzel Nuñez del Toro, Roberta Evangelista, Barbara Imbrosci, Aarti Swaminathan, Dietmar Schmitz, Nikolaus Maier, and Richard Kempter

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The referee reports are copied at the end of this email.

Please address all the points raised and incorporate all requested revisions or explain in your Response to Referees why a change has not been made. We hope you will find the comments helpful and that you will be able to return your revised manuscript within 4weeks. If you require longer than this, please contact journal staff: jp@physoc.org.

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If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

Katalin Toth
Senior Editor
The Journal of Physiology

REQUIRED ITEMS

- You must start the Methods section with a paragraph headed Ethical Approval. A detailed explanation of journal policy and regulations on animal experimentation is given in Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818). A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: https://physoc.onlinelibrary.wiley.com/hub/animal-experiments. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015), including an ethics approval reference number. The Methods section must contain a statement about access to food, water and housing, details of the anaesthetic regime: anaesthetic used, dose and route of administration, and method of killing the experimental animals.

EDITOR COMMENTS

Reviewing Editor:

Methods Details:
To comply with our animal ethics policy, please provide in the Methods section: (1) a statement regarding origin and source of animals and (2) a statement about animals' access to food/water.

Both reviewers are generally happy with the revised version of this manuscript. While one of the reviewer is satisfied with the responses by the authors to the previous round of review, he/she wishes that several of these points be explicitly incorporated in the discussion of the manuscript. I see this exercise of transparency as laudable and reasonable (and easily addressed by the authors).

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The authors should therefore incorporate these graphs as part of the main figures.

REFEREE COMMENTS

Referee #1:

The authors have convincingly answered to the points raised by the reviewer.

Referee #2:

The authors have responded in detail to concerns. A few of the clarifications that are in the response should also be included in the revised manuscript.

1) Geometry of the slice preparation: the authors indicate that spontaneous population activity can be observed and cite studies using ventral, horizontal slices (as well as some irrelevant studies such as 1 mm thick slice preparations). The current study uses horizontal slices, but the ventral nature of these slices can't be determined with certainty because the ex vivo slicing coordinates are referenced to the bregma, a landmark that no longer exists during slicing. If these are ventral slices, please say so.

2) Geometry of the recording system: the authors point out in response to the question of slice trauma that this commercial system is comprised of planar electrodes. They further specify that there are perforations in the electrode surface that allow negative pressure to fix the slice. How these perforations and the negative pressure alter the slice is not described. Further, the question of R1 re: propagation to CA1 is answered by the physical limitations of the electrode array. These issues would be much more clear to readers if an actual photograph of the experimental preparation were included in the first figure, or even a supplemental figure.

3) Response to 1b, the impact of slice trauma on acute recordings: This is a reasonable response, but the caveats need to be included in the discussion because the question will occur to many future readers.

4) Comment 2b: the combination of inhibitory and excitatory synaptic conductances alter membrane potential responses and measures of conduction velocity. This problem is only partly addressed by holding near the estimated inhibitory reversal potential. An EPSP in the absence of a shunting inhibitory conductance will be larger and have a much earlier onset than an EPSP in the presence of a shunting conductance. The mirror-image EPSC and IPSC shown in green in Figure 1A illustrate how inextricably the excitatory and inhibitory conductances are mixed: changing the holding potential has no effect other than to flip the waveform (no components of the waveform disappear, as would be predicted if holding near the reversal potential of one component actually cancelled that component). The issues addressed in comments 2a and b are inherent to all acute slice electrophysiology studies, as the authors imply by their citations in the rebuttal. But these issues should be addressed in the discussion, not only in the response to the first (or second) reader of their paper. Many labs are moving to voltage imaging in vivo; the resolution is different, and the results will be different. For the continued relevance of this study, these issues should be addressed in the discussion.

END OF COMMENTS

1st Confidential Review **24-Apr-2024**

Second resubmission (July 2024)

EDITOR COMMENTS

Reviewing Editor:

[...] Both reviewers are generally happy with the revised version of this manuscript. While one of the reviewers is satisfied with the responses by the authors to the previous round of review, he/she wishes that several of these points be explicitly incorporated in the discussion of the manuscript. I see this exercise of transparency as laudable and reasonable (and easily addressed by the authors).

We would like to once again thank both reviewers and the Reviewing Editor for their constructive and supportive feedback on our current work. Below, you will find our point-by-point responses to the remaining questions and issues raised. We have incorporated the requested changes and hope our manuscript now meets the final requirements for acceptance. We sincerely appreciate the opportunity to publish in *The Journal of Physiology*.

On behalf of all co-authors,
Sincerely,

Natalie Schieferstein,
Nikolaus Maier,
Richard Kempter

EDITOR COMMENTS

Reviewing Editor:

Methods Details:

To comply with our animal ethics policy, please provide in the Methods section: (1) a statement regarding origin and source of animals and (2) a statement about animals' access to food/water.

Response: We have updated the 'Subjects' section as follows: *"Inbred mice used in this study were obtained from the Experimental Medicine Research Facilities (FEM) of Charité-Universitätsmedizin Berlin and were housed with ad libitum access to food and water. A total of 29 male C57BL/6N mice, aged 3 to 9 weeks, were analyzed."*

[...] While one of the reviewers is satisfied with the responses by the authors to the previous round of review, he/she wishes that several of these points be explicitly incorporated in the discussion of the manuscript. I see this exercise of transparency as laudable and reasonable (and easily addressed by the authors).

Response: We have incorporated all points in the manuscript; see also our detailed response below.

Also, note that The Journal of Physiology does not publish supplemental or appendix figures.
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https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#suppinfo
The authors should therefore incorporate these graphs as part of the main figures.

Response: We have incorporated the gist of the two appendix figures in the main text.
1) To summarize the previous Fig. S1, we have added a paragraph at the end of the 'Results' Section, which reads: "*The age of the mice analyzed in this study ranged from 3 to 9 weeks. It has been shown that the occurrence of SPW-R events and the ripple frequency are stable from the third week of age onwards (Buhl and Buzsáki, 2005; Wong et al., 2005; Buzsáki, 2015). In a subset of whole-cell experiments, we tested for possible correlations between SPW propagation speed/directionality, and age. Neither parameter showed a clear correlation. More specifically, a linear regression of median SPW propagation slope vs age resulted in r=0.10, p=0.618 for cEPSCs (n=28 slices) and r=-0.07, p=0.725 for cIPSCs (n=30 slices); a linear regression of median SPW*

propagation speed vs age resulted in r=0.38, p=0.045 for cEPSCs and r=-0.09, p=0.635 for cIPSCs. We conclude that the age of mice included in our study had no substantial effect on our results."

2) We have included the information previously conveyed in Fig. S2 as part of the section "Materials and Methods>Data analysis: Combined LFP/whole-cell recordings>Propagation analysis of SPW-associated synaptic activity". In detail, we now specify: *"[...] SPW-associated cPSCs in single cells were excluded from further analysis if at least one of the following criteria was met: (1) cPSCs had a polarity that did not match the direction of the driving force at the particular holding potential; (2) the recording was too noisy (peak in cPSC was smaller than the baseline current plus 2 SD); (3) the threshold-linear fit of the rise time failed (mean squared error larger than 0.1 pA^2); or (4) the rise time ($t_{peak} - t_0$) was longer than 30 ms. For SPW events with accepted cPSCs in all cells (7862/9603 events, i.e. 81.87%), the propagation delay was determined as described above. Three recording sessions (two or three cells recorded simultaneously at the same holding potential) were excluded from analysis, because they contained less than 25 admissible SPW events (slice ID:19, cIPSC recording; slice ID:21, both cIPSC and cEPSC recordings). In slices ID:9, 12, and 17 only cIPSCs were recorded."*

REFEREE COMMENTS

Referee #2:

The authors have responded in detail to concerns. A few of the clarifications that are in the response should also be included in the revised manuscript.

Response: Again, we wish to thank this reviewer for their detailed and constructive feedback on our manuscript. We agree that the inclusion of the suggested points is reasonable and of potential value to colleagues working in the field.

1) Geometry of the slice preparation: the authors indicate that spontaneous population activity can be observed and cite studies using ventral, horizontal slices (as well as some irrelevant studies such as 1 mm thick slice preparations). The current study uses horizontal slices, but the ventral nature of these slices can't be determined with certainty because the ex vivo slicing coordinates are referenced to the bregma, a landmark that no longer exists during slicing. If these are ventral slices, please say so.

Response: We have now specified the anatomical position of the hippocampal slices used in this study. The revised sentences can be found in the section "Materials and Methods>Slice preparation" and now read: *"[...] After ≈ 5 min of recovery, horizontal slices (350–400 µm thick) were cut using a vibratome (VT1200S, Leica, Germany) starting from the ventral pole of the hippocampus. Slices used in this study are from the ventral to middle portion of the hippocampus, and post-hoc alignment of their images with anatomical references confirms their position in the range of [-2.80, -3.76] mm with respect to bregma, corresponding to Figures 156 to 146 in Franklin and Paxinos (2007). Subsequently, the slices were stored in a holding chamber for recovery..."*

2) Geometry of the recording system: the authors point out in response to the question of slice trauma that this commercial system is comprised of planar electrodes. They further specify that there are perforations in the electrode surface that allow negative pressure to fix the slice. How these perforations and the negative pressure alter the slice is not described. Further, the question of R1 re: propagation to CA1 is answered by the physical limitations of the electrode array. These issues would be much more clear to readers if an actual photograph of the experimental preparation were included in the first figure, or even a supplemental figure.

Response: Thank you for this suggestion. The effect of perforations is described in the manuscript (section "Materials and Methods>Electrophysiology>MEA recordings"): *"A second chamber below the membrane was pressurized to a slight vacuum of ≈18-20 mbar, which exerted negative pressure on the slice via the perforation that resulted in (1) improved contact between the slice and the electrodes and (2) increased flow of ACSF from the recording chamber through the slice. The perforation of MEA membranes has been demonstrated to improve tissue oxygenation and signal quality (Egert et al., 2005)."*

To better illustrate the actual experimental conditions, we have now presented the experimental preparation in a photograph in Fig. 1*A* in a zoomed-out version (revised Fig. 1*Ac*), as suggested by this reviewer. We now also display details of the experimental chamber and the perforation (revised Fig. 1*Aa,Ab*).

3) Response to 1b, the impact of slice trauma on acute recordings: This is a reasonable response, but the caveats need to be included in the discussion because the question will occur to many future readers.

Response: We have now included the mentioned arguments in our manuscript. However, we feel that this addition addresses technical aspects rather than contributing to the discussion of our present findings. At the discretion of the referee and/or the editor, we decided to place it in the paragraph "Slice preparation" in the Materials and Methods section, which now reads: *"[...] It should be noted that the slicing procedure in ice-cold ACSF can induce a transient loss of spines (Kirov et al., 2004); spines were shown to re-emerge within 30 minutes, and spine and synapse number reached a plateau within 2 hours. Our experiments were performed within 2–7 hours after slicing."*

4) Comment 2b: the combination of inhibitory and excitatory synaptic conductances alter membrane potential responses and measures of conduction velocity. This problem is only partly addressed by holding near the estimated inhibitory reversal potential. An EPSP in the absence of a shunting inhibitory conductance will be larger and have a much earlier onset than an EPSP in the presence of a shunting conductance. The mirror-image EPSC and IPSC shown in green in Figure 1A illustrate how inextricably the excitatory and inhibitory conductances are mixed: changing the holding potential has no effect other than to flip the waveform (no components of the waveform disappear, as would be predicted if holding near the reversal potential of one component actually cancelled that component). The issues addressed in comments 2a and b are inherent to all acute slice electrophysiology studies, as the authors imply by their citations in the rebuttal. But these issues should be addressed in the discussion, not only in the response to the first (or second) reader of their paper. Many labs are moving to voltage imaging in vivo; the resolution is different, and the results will be different. For the continued relevance of this study, these issues should be addressed in the discussion.

Response: There is evidence against the statement that "changing the holding potential has no effect other than to flip the waveform." First, SPW-R associated excitatory and inhibitory synaptic currents differ in their amplitudes — inhibitory ripple-associated PSCs are larger by up to an order of magnitude, with cEPSCs measuring several hundred picoamperes and cIPSCs reaching up to a few nanoamperes, as shown in the present Figure 4*B*. Additionally, the kinetics of ripple-linked cEPSCs and cIPSCs differ, as investigated in individual PSCs (see, e.g., Fig. 4*C*; see also Swaminathan et al., 2018 for CA3 PCs; Maier et al., 2011 for CA1 PCs). Finally, the onset timing of both components is different: cEPSCs lead, and cIPSCs follow (Maier et al., 2011; Swaminathan et al., 2018).

To align with this complex picture, we decided to modify the Graphical Abstract. In its previous version, we simply mirrored the excitatory component to represent the inhibitory component for graphical simplicity. However, this oversimplification might incorrectly suggest an equivalence of both components, differing only in polarity. We apologize for any confusion this may have caused!

We do agree with the reviewer that discussing these issues will add valuable information. Therefore, we have incorporated snippets from our response to their previous comment 2b in the Materials and Methods. The corresponding paragraph now reads ("Materials and Methods>Propagation analysis of SPW-associated synaptic activity"): *"We note that this analysis of the propagation of SPW-associated cPSCs, which we measured using voltage-clamp recordings, is largely unaffected by fluctuations in the reversal potentials. The reversal potentials may fluctuate during the span of a SPW-R event due to voltage-dependent membrane conductances. Our selection of the intracellular solution, containing TEA, Cs^+, and QX-314, substantially reduces K^+-based conductances and Na^+-driven spiking activities (Monier et al., 2008; Simonnet et al., 2021). Furthermore, the above described read-out parameter ("onset time") is robust against variations in the amplitude of the cPSCs."*

Dear Dr Kempter,

Re: JP-RP-2024-285671R2 "Propagation of sharp wave-ripple activity in the mouse hippocampal CA3 subfield in vitro" by Natalie Schieferstein, Ana Itzel Nuñez del Toro, Roberta Evangelista, Barbara Imbrosci, Aarti Swaminathan, Dietmar Schmitz, Nikolaus Maier, and Richard Kempter

We are pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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Katalin Toth
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

The authors provided a detailed rebuttal and have made changes to the manuscript accordingly. I went carefully through the rebuttal: while some disagreement with the reviewer is expressed, I see it to be well-supported and reasonable. I am happy with the this version of the manuscript for publication.
