# **Point-by-Point Replies for Editor and Reviewers**

**Hippocampal Neural Fluctuation between Memory Encoding and Retrieval States During a Working Memory Task in Humans**

**(Manuscript. Number.: HELIYON-D-23-51876R1)**

We appreciate the Editor and Reviewers' insightful comments and suggestions, which have been invaluable in enhancing our manuscript. We have conducted revisions to address all the raised concerns. *The original comments from the Editor and Reviewers are presented in inclined gray text for reference.* Our responses are provided in blue. Text that has been added or modified to the revised manuscript is highlighted in yellow.

# **Comments to Dr. Davide Rivolta, Associate Editor – Neuroscience, Heliyon**

## **Dr. Davide’s Comments 1:**

*Dear Dr Watanabe,*

*Thank you for submitting your manuscript to Heliyon. We have completed the review of your manuscript and a summary is appended below. The reviewers recommend major revisions are required before publication can be considered. If you are able to address all reviewer comments in full, I invite you to resubmit your manuscript. We ask that you respond to each reviewer comment by either outlining how the criticism was addressed in the revised manuscript or by providing a rebuttal to the criticism.*

*This should be carried out in a point-by-point fashion as illustrated here: https://www.cell.com/heliyon/guide-for-authors#Revisions.*

**Authors’ Response:**

We greatly appreciate Dr. Davide for organizing this review process and thank the reviewers for their detailed and constructive feedback. We have carefully revised the manuscript, addressing each of the concerns raised, and have incorporated the reviewers' valuable suggestions. We believe these revisions have significantly improved the manuscript and hope it now meets the publication standards of ***Heliyon***.

## **Dr. Davide’s Comments 2:**

*We also request you to ensure the following about data availability.*

*While first submitting your manuscript, you were asked two questions regarding data availability. The questions and your responses to them are as follows,*

*Additional Information*

*1. Yusuke Watanabe*

*Question Response*

*Data Availability*

*Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.*

*Has data associated with your study been deposited into a publicly available repository?*

*Yes*

*Please provide the name of the repository and the accession number here.*

*as follow-up to "Data Availability*

*Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication.*

*Has data associated with your study been deposited into a publicly available repository?*

*" The data is available on G-Node (https://doi.gin.g-node.org/10.12751/g-node.d76994/). The*

*Please add these responses to the ‘Data availability statement’ section of your manuscript. If your manuscript doesn’t contain a ‘Data availability statement’ section, please add one right before the ‘References’ section and then include these responses therein.*

**Authors’ Response:**

Thank you very much for providing the ***Heliyon*** standard format. We have moved the “Data availability statement” section right before the References section as follows:

Lines *XX–XX*

~~Data and code availability~~

~~The data is available on G-Node (https://doi. gin.g-node.org/10.12751/g-node.d76994/). The source code is available on GitHub (https://github.com/yanagisawa-lab/ hippocampal-neural-fluctuations-during-a-WM-task-in-humans).~~

*Lines XX–XX*

Data availability statement

The data is available on G-Node (https://doi. gin.g-node.org/10.12751/g-node.d76994/). The source code is available on GitHub (https://github.com/yanagisawa-lab/ hippocampal-neural-fluctuations-during-a-WM-task-in-humans).

## **Dr. Davide’s Comments 3:**

*Furthermore, please note that Heliyon now uses a Numbered reference style. Please update the references in your manuscript accordingly, if necessary.*

**Authors’ Response:**

We appreciate you informing us about ***Heliyon***’s adoption of the numbered reference style. We have updated the references in our manuscript accordingly to align with this standard.

## **Dr. Davide’s Comments 4:**

*To allow the editors and reviewers to easily assess your revised manuscript, we also ask that you upload a version of your manuscript highlighting any revisions made. You may wish to use Microsoft Word's Track Changes tool or, for LaTeX files, the latexdiff Perl script (https://ctan.org/pkg/latexdiff).To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/heliyon/, and navigate to the "Submissions Needing Revision" folder.*

**Authors’ Response:**

Thank you for your guidance on how to effectively highlight revisions in our manuscript. We have utilized the latexdiff Perl script as suggested and have attached both the revised manuscript and the difference-visualized PDF file to our submission.

## **Dr. Davide’s Comments 5:**

*Please note that our ethics requirements are now updated. Please choose all applicable statements in our ethics declarations list (available here: https://www.cell.com/heliyon/ethics) and include them as a complete ethics statement in the declarations section at the end of your manuscript.*

**Authors’ Response:**

**Thank you for notifying us about the updated ethics requirements. We have carefully reviewed the list provided and included a comprehensive ethics statement in the declarations section of our manuscript.**

**Lines XX–XX**

**Ethics Declarations**

**All study participants provided their written informed consent, subsequent to the approval from the pertinent institutional ethics review board (Kantonale Ethikkom-mission Zürich, PB 2016–02055).**

## **Dr. Davide’s Comments 6:**

*Your revision due date is May 22, 2024.We understand that the COVID-19 pandemic may well be causing disruption for you and your colleagues. If that is the case for you and it has an impact on your ability to make revisions to address the concerns that came up in the review process, please reach out to us.*

*If you need additional time to address the concerns that came up in the review process, please let us know so we can discuss a plan for moving your paper forward.*

*I look forward to receiving your revised manuscript.*

*Research Elements (optional)*

*This journal encourages you to share research objects - including your raw data, methods, protocols, software, hardware and more – which support your original research article in a Research Elements journal. Research Elements are open access, multidisciplinary, peer-reviewed journals which make the objects associated with your research more discoverable, trustworthy and promote replicability and reproducibility. As open access journals, there may be an Article Publishing Charge if your paper is accepted for publication. Find out more about the Research Elements journals at https://www.elsevier.com/authors/tools-and-resources/research-elements-journals?dgcid=ec\_em\_research\_elements\_email.*

*Kind regards,*

*Davide Rivolta, Ph.D.*

*Associate Editor - Neuroscience*

*Heliyon*

*Editor and Reviewer comments:*

*Please note that manuscripts with inadequate language quality will not be accepted in the journal. If editors and / or reviewers indicate that language revisions are required for your manuscript, we strongly encourage using a professional language editing service. Elsevier's Language Editing services provides professional and prompt editing of scientific language for research submissions (https://webshop.elsevier.com/language-editing-services/language-editing/). All manuscripts edited with Elsevier's Language Editing services are accompanied by a certificate that may be submitted to the journal as proof for language editing. Poor language may lead to rejection of your manuscript even at the revision stage.*

**Authors’ Response:**

**Thank you, Dr. Rivolta, for informing us of the revision due date and for understanding the potential disruptions caused by the COVID-19 pandemic. We have diligently worked on addressing the feedback from you and the reviewers. Additionally, we acknowledge the suggestions regarding Research Elements and language quality improvements. We are exploring the use of Elsevier's Language Editing services to ensure the manuscript meets the required standards.**

*Reviewers Ansewrs to Questionnairs*

*Reviewer's Responses to Questions*

*\*Is the manuscript scientifically sound and well presented?*

*Reviewer 1:*

*Yes*

*Reviewer 2:*

*Partly*

*Arethestudydesign,methodologyandstatisticalanalysesrobust?</p><p>Reviewer<ul><li>Partly</li></ul><p> </p><p>Reviewer<ul><li>Partly</li></ul><p> </p><hr/><p>Aretheconclusionsinalignmentwiththeresultsanddiscussion?</p><p>Reviewer<ul><li>Yes</li></ul><p> </p><p>Reviewer<ul><li>Partly</li></ul><p> </p><hr/><p>Are the study design, methodology and statistical analyses robust?*

*Reviewer 1:*

*Partly*

*Reviewer 2:*

*Partly*

*Are the conclusions in alignment with the results and discussion?*

*Reviewer 1:*

*Yes*

*Reviewer 2:*

*Partly*

*Is the revised manuscript scientifically sound and have all concerns been addressed? (Only for revisions)*

*Reviewer 1:*

*Not Applicable (this is not a revised manuscript)*

*Reviewer 2:*

*Partly*

*$$Are there any new concerns in the revised manuscript? (Only for revisions)*

*Reviewer 1:*

*Not Applicable (this is not a revised manuscript)*

*Reviewer 2:*

*Yes*

*Could the manuscript benefit from language editing?*

*Reviewer 1: No*

*Reviewer 2: No*

# **Comments to Reviewers**

## **Reviewer 1:**

### **Reviewer 1’s Comments 1:**

*Reviewer 1: Response is required. Please include your detailed assessment of the manuscript. If you are reviewing a revision, please also indicate if any additional revisions are needed.*

*This is an interesting study. I have some comments as follows,*

**Authors’ Response:**

**We appreciate Reviewer 1’s positive feedback on our manuscript. We are eager to address your comments and suggestions to further improve our study.**

### **Reviewer 1’s Comments 2:**

*The introduction of the study should situate the current research in the context of existing literature, and clearly address the motivation. For example,*

*1)the authors mentioned sharp-wave ripples (SWRs) are associated with memory consolidation, recall, and neural plasticity, all of which are from long-term memory functions. Then, what's the rationale for investigating SWRs in working memory (WM)?*

**Authors’ Response:**

**Thank you for your feedback. We agree that SWRs have been primarily associated with long-term memory functions. However, we believe that their role in working memory (WM) has been underexplored due to limitations in experimental settings. Access to the human hippocampus has been restricted by ethical and safety considerations, whereas animal studies often face challenges due to the low temporal resolution of tasks, making it difficult to precisely determine when the animal acquires and utilizes WM information. Additionally, SWR detection has been limited to “offline” instances, when the animals speed are often less than 4 cm/s, partially due to noise contamination, which inheretently have inhibited studying the involvement of SWRs during navigation. These constraints complicate the identification of SWRs during WM tasks. While some studies have linked SWRs to WM tasks in rodents, the SWR detection was performed only when animals are not walking. By addressing these issues in our study, we aim to provide a clearer understanding of the potential involvement of SWRs in WM processes.**

**In response, we have revised the Introduction section as follows:**

**1. Introduction**

**Working memory (WM) is crucial in everyday life; however, its neural mechanism has yet to be fully elucidated. Speciﬁcally, the hippocampus’s involvement in WM processing, a pivotal region for memory, is the subject of ongoing research [1, 2, 3, 4, 5, 6, 7, 8, 9]. Understanding the hippocampus’ role in working memory is instrumental in deepening our comprehension of cognitive processes and could potentially enhance cognitive abilities. Current evidence suggests that transient, synchronized oscillations, termed sharp-wave ripples (SWRs) [10], are associated with various cognitive func- tions. SWRs have traditionally been linked with long-term memory func- tions such as memory replay [11, 12, 13, 14, 15], memory consolidation [16, 17, 18, 19], memory recall [20, 21, 22], and neural plasticity [23, 24]. However, only a subset of studies has investigated the role of SWRs in WM tasks [25, 26]. This gap in our understanding motivates the current study to further investigate the potential involvement of SWRs in WM, particularly given their fundamental computational manifestation in hippocampal processing. Recent studies have found that low-dimensional representations in hippocampal neurons can explain WM task performances. Speciﬁcally, the ﬁring patterns of place cells [27, 28, 29, 30, 31], found in the hippocampus, have been identiﬁed within dynamic, nonlinear three-dimensional hyperbolic spaces in rats [32]. Additionally, grid cells in the entorhinal cortex (EC), which is the main pathway to the hippocampus [33, 34, 35], exhibited a toroidal geometry during exploration in rats [36]. However, these existing studies predominantly focus on spatial navigation in rodents, presenting several limitations. First, the temporal resolution of navigation tasks is insuﬃcient, obscuring the precise timing of memory acquisition and recall. Second, the presence of noise in signals recorded during rodent movement complicates the detection of SWRs [37]. Third, the generalization to humans and tasks other than spatial navigation remains unclear. Given these limitations, it is crucial to explore SWRs in a controlled, less noisy environment to better understand their potential role in WM tasks in humans.**

**Considering these factors, this study investigates the hypothesis that hippocampal neurons in humans exhibit low-dimensional neural trajectories (NTs) that depend on WM load, particularly during SWR periods. To test this hypothesis, we employed a dataset of patients performing an eight- second Sternberg task (1 s for ﬁxation, 2 s for encoding, 3 s for maintenance, and 2 s for retrieval) with high temporal resolution. Intracranial electroen- cephalography (iEEG) signals within the medial temporal lobe (MTL) were recorded for these patients [38]. To investigate low-dimensional NTs, we uti- lized Gaussian-process factor analysis (GPFA), an established method for analyzing neural population dynamics [39].**

### **Reviewer 1’s Comments 3 - 1:**

*2)what's the motivation of exploring neural representations or trajectories (NT) during SWR periods?*

**Authors’ Response:  
  
Thank you for your inquiry regarding the motivation for exploring neural trajectories (NT) during sharp-wave ripple (SWR) periods.**

**The impetus for investigating NT during SWR periods is primarily due to limitations observed in existing studies which focus on spatial navigation in rodents. These limitations include insufficient temporal resolution, significant noise in signal detection during rodent movement, and the challenges of generalizing these findings to humans and non-navigation tasks. Given these challenges, exploring SWRs in a controlled, less noisy environment is crucial for understanding their potential role in working memory (WM) tasks in humans.**

**Accordingly, we have amended the Introduction section as follows:**

**Lines XX-XX  
  
However, these existing studies predominantly focus on spatial navigation in rodents, presenting several limitations. First, the temporal resolution of navigation tasks is insuﬃcient, obscuring the precise timing of memory acquisition and recall. Second, the presence of noise in signals recorded during rodent movement complicates the detection of SWRs [37]. Third, the generalization to humans and tasks other than spatial navigation remains unclear. Given these limitations, it is crucial to explore SWRs in a controlled, less noisy environment to better understand their potential role in WM tasks in humans.**

### **Reviewer 1’s Comments 3-2:**

*Has this metric been utilized to study working memory or cognitive functions?*

**Authors’ Response:**

**Thank you for your inquiry regarding the application of Gaussian-Process Factor Analysis (GPFA) in studying working memory and other cognitive functions. We have thoroughly reviewed the literature and identified multiple instances where GPFA has been effectively utilized in cognitive research.**

**Foundational Work and Visualizations:**

**GPFA was first introduced by Yu et al., in 2009, a study that has been cited extensively, indicating its importance in neural data analysis. The foundational paper provides intuitive visualizations on how GPFA extracts neural trajectories:**

**Figure 2: View Figure 2**

**Figure 8: View Figure 8**

**Software and Tools:**

**We utilized the elephant package for the GPFA calculations, which provides a comprehensive and practical tutorial: elephant GPFA tutorial.**

**Relevant Literature:**

**We have incorporated the following key studies into our manuscript that demonstrate the versatility and effectiveness of GPFA in analyzing neural dynamics across various contexts:**

**Yu, B. M. et al. (2009): GPFA was used to extract dynamic patterns from neurons' spike train data in single trials.**

**Churchland, M. M. et al. (2010): GPFA demonstrated reduced neural variability upon stimulus onset, indicating synchronized cortical responses.**

**Lin, D. et al. (2011): GPFA identified specific neural activity patterns linked to aggression in mice.**

**Churchland, M. et al. (2012): During reach tasks, GPFA revealed dynamic neural patterns suggesting continuous evolution of neural state.**

**Ecker, A. S. et al. (2014): GPFA analyzed noise correlations within macaque visual cortex, showing how external states influence internal dynamics.**

**Kao, J. C. et al. (2015): Demonstrated GPFA's application in decoding neural dynamics for brain-machine interface improvements.**

**Gallego, J. A. et al. (2017): Used GPFA to map motor cortex activity onto a lower-dimensional manifold.**

**Wei, Z. et al. (2019): GPFA revealed how population dynamics in premotor cortex are organized on a trial-by-trial basis.**

**Kim, J. et al. (2023): Explored the dynamics of cortical-hippocampal interactions during motor tasks, indicating complex coordination.**

**These references underscore GPFA’s significant role in advancing our understanding of neural mechanisms underlying cognitive functions, including working memory.**

**Accodingly, we have updated our references as follows:**

**Lines XX–XX:**

**To investigate low-dimensional NTs, we uti-**

**lized Gaussian-process factor analysis (GPFA), an established method for**

**analyzing neural population dynamics [39, 40, 41, 42, 43, 44, 45, 46, 47].**

**Lines XX–XX:**

**[39] B. M. Yu, J. P. Cunningham, G. Santhanam, S. I. Ryu, K. V. Shenoy,**

**M. Sahani, Gaussian-Process Factor Analysis for Low-Dimensional**

**Single-Trial Analysis of Neural Population Activity, Journal of Neu-**

**rophysiology 102 (1) (2009) 614–635. doi:10.1152/jn.90941.2008.**

**URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2712272/**

**[40] M. M. Churchland, B. M. Yu, J. P. Cunningham, L. P. Sugrue, M. R.**

**Cohen, G. S. Corrado, W. T. Newsome, A. M. Clark, P. Hosseini, B. B.**

**Scott, D. C. Bradley, M. A. Smith, A. Kohn, J. A. Movshon, K. M.**

**Armstrong, T. Moore, S. W. Chang, L. H. Snyder, S. G. Lisberger,**

**N. J. Priebe, I. M. Finn, D. Ferster, S. I. Ryu, G. Santhanam, M. Sahani,**

**K. V. Shenoy, Stimulus onset quenches neural variability: a widespread**

**cortical phenomenon, Nature neuroscience 13 (3) (2010) 369–378. doi:**

**10.1038/nn.2501.**

**URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2828350/**

**[41] D. Lin, M. P. Boyle, P. Dollar, H. Lee, E. S. Lein, P. Perona, D. J.**

**Anderson, Functional identification of an aggression locus in the mouse**

**hypothalamus, Nature 470 (7333) (2011) 221–226, publisher: Nature**

**Publishing Group. doi:10.1038/nature09736.**

**URL https://www.nature.com/articles/nature09736**

**[42] M. Churchland, J. Cunningham, M. Kaufman, J. Foster, P. Nuyujukian,**

**S. Ryu, K. Shenoy, Neural population dynamics during reaching, Nature**

**487 (7405) (2012) 51–56. doi:10.1038/nature11129.**

**URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3393826/**

**[43] A. S. Ecker, P. Berens, R. J. Cotton, M. Subramaniyan, G. H. Denfield,**

**C. R. Cadwell, S. M. Smirnakis, M. Bethge, A. S. Tolias, State depen-**

**dence of noise correlations in macaque primary visual cortex, Neuron**

**82 (1) (2014) 235–248. doi:10.1016/j.neuron.2014.02.006.**

**URL** [**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990250/**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990250/)

**[44] J. C. Kao, P. Nuyujukian, S. I. Ryu, M. M. Churchland, J. P. Cunning-**

**ham, K. V. Shenoy, Single-trial dynamics of motor cortex and their ap-**

**plications to brain-machine interfaces, Nature Communications 6 (2015)**

**7759. doi:10.1038/ncomms8759.**

**[45] J. A. Gallego, M. G. Perich, L. E. Miller, S. A. Solla, Neural Man-**

**ifolds for the Control of Movement, Neuron 94 (5) (2017) 978–984.**

**doi:10.1016/j.neuron.2017.05.025.**

**URL**

**https://www.sciencedirect.com/science/article/pii/**

**S0896627317304634**

**[46] Z. Wei, H. Inagaki, N. Li, K. Svoboda, S. Druckmann, An orderly single-**

**trial organization of population dynamics in premotor cortex predicts**

**behavioral variability, Nature Communications 10 (1) (2019) 216, pub-**

**lisher: Nature Publishing Group. doi:10.1038/s41467-018-08141-6.**

**URL https://www.nature.com/articles/s41467-018-08141-6**

**[47] J. Kim, A. Joshi, L. Frank, K. Ganguly, Cortical–hippocampal cou-**

**pling during manifold exploration in motor cortex, Nature 613 (7942)**

**(2023) 103–110, publisher: Nature Publishing Group. doi:10.1038/**

**s41586-022-05533-z.**

**URL https://www.nature.com/articles/s41586-022-05533-z**

### **# Reviewer 1’s Comments 3-3:**

*how the findings of the present study compare with past research?*

**Authors’ Response:**

**Thank you for your question regarding how our findings compare with past research.**

**Previous studies have indeed acknowledged the involvement of the hippocampus in working memory (WM) tasks. However, many of these studies were constrained by the temporal resolution of their methods, limiting their ability to capture the precise dynamics of memory processing. Our study advances this understanding by employing a dataset with a higher temporal resolution of 1 second, making it the first to explicitly delineate the neural fluctuations between the encoding and retrieval states during a WM task.**

**This significant advancement provides detailed insights into the temporal dynamics of hippocampal activity, offering a clearer picture of how specific phases of memory processing are supported by neural mechanisms. Our findings address a crucial gap in the literature and may serve as a foundation for future research exploring the complexities of neural activity during different stages of working memory tasks.**

**According to this discussion, we have revised our manuscript as follows for better communication with readers.**

### **Lines XX–XX**

**…**

### **Reviewer 1’s Comments 4:**

*In the methods section, a more detailed description is warranted. For instance, the frequency range defining SWRs is set at 80-140 Hz, different from previous studies that identified sharp-wave ripples in higher frequency bands (e.g., 140-200 Hz, in "SPW-Rs are fast (140-200 Hz) oscillations in field potential recordings that are superimposed on a slow field potential transient", see doi:https://doi.org/10.1038/nn1571, Nature, 2005). The authors should explain the criteria for selecting this frequency range.*

**Authors’ Response:**

**Thank you for your insightful question regarding the frequency range used to define SWRs in our study.**

**The referenced paper (doi:https://doi.org/10.1038/nn1571, Nature, 2005) has investigated the stimuli-induced / spontaneous SWRs in rats. However, in humans, researchers believe that the frequency band for SWRs shifted to lower bands such as 80-140 Hz, which we followed in this study. This band shift stems from the observation of wavelet-transformed signals.**

**In fact, a concensus paper in the field related to hippocampal SWRs [49] mentions as follows:**

**```SPW-R frequency band criterion for rodents (100 to 250 Hz) is generally higher than for monkeys (95 to 250 Hz) or humans (70–250 Hz, most use 80–150 Hz bandpass filters; Supplementary Table S1).```**

**Thanks to your enquiry, we have successfully updated the method section as follows:**

**Lines: XX–XX**

**SWR detection was carried out using a published tool (https://github.**

**com/Eden-Kramer-Lab/ripple\_detection) [50], with the bandpass range**

**adjusted to 80–140 Hz for humans [21, 22, 49], unlike the original 150–250**

**Hz range typically applied to rodents [10].**

**Reference:**

**[21] Y. Norman, E. M. Yeagle, S. Khuvis, M. Harel, A. D. Mehta, R. Malach,**

**Hippocampal sharp-wave ripples linked to visual episodic recollection in**

**humans, Science 365 (6454) (2019) eaax1030. doi:10.1126/science.**

**aax1030.**

**URL https://www.sciencemag.org/lookup/doi/10.1126/science.**

**aax1030**

**[22] Y. Norman, O. Raccah, S. Liu, J. Parvizi, R. Malach, Hippocampal**

**ripples and their coordinated dialogue with the default mode network**

**during recent and remote recollection, Neuron 109 (17) (2021) 2767–**

**2780.e5, publisher: Elsevier. doi:10.1016/j.neuron.2021.06.020.**

**URL**

[**https://www.cell.com/neuron/abstract/S0896-6273(21)**](https://www.cell.com/neuron/abstract/S0896-6273(21))

**[49] A. A. Liu, S. Henin, S. Abbaspoor, A. Bragin, E. A. Buffalo, J. S. Farrell,**

**D. J. Foster, L. M. Frank, T. Gedankien, J. Gotman, J. A. Guidera, K. L.**

**Hoffman, J. Jacobs, M. J. Kahana, L. Li, Z. Liao, J. J. Lin, A. Losonczy,**

**R. Malach, M. A. van der Meer, K. McClain, B. L. McNaughton, Y. Nor-**

**man, A. Navas-Olive, L. M. de la Prida, J. W. Rueckemann, J. J. Sakon,**

**I. Skelin, I. Soltesz, B. P. Staresina, S. A. Weiss, M. A. Wilson, K. A.**

**Zaghloul, M. Zugaro, G. Buzsáki, A consensus statement on detection of**

**hippocampal sharp wave ripples and differentiation from other fast os-**

**cillations, Nature Communications 13 (1) (2022) 6000, number: 1 Pub-**

**lisher: Nature Publishing Group. doi:10.1038/s41467-022-33536-x.**

**URL** [**https://www.nature.com/articles/s41467-022-33536-x**](https://www.nature.com/articles/s41467-022-33536-x)

**[50] K. Kay, M. Sosa, J. E. Chung, M. P. Karlsson, M. C. Larkin, L. M.**

**Frank, A hippocampal network for spatial coding during immobility and**

**sleep, Nature 531 (7593) (2016) 185–190. doi:10.1038/nature17144.**

### **Reviewer 1’s Comments 5:**

*In the results, while the authors showed their reasons focusing on the CA1 subregion of the hippocampus, what were the results for the other subregions? If these results were negative in other subregions, they could serve as a control to underscore the functions of CA1.*

**Authors’ Response:**

**A diagram of a graph

Description automatically generatedWe greatly appreciate your insights regarding the specificity of the CA1 region and acknowledge the importance of addressing findings in other subregions of the hippocampus. However, precise localization of recording electrodes within the hippocampal subregions (e.g., CA1, CA2, CA3, CA4, Dentate Gyrus) or confirming the electrode tip's placement within the hippocampal body presents significant challenges in human studies, primarily due to the absence of postmortem histological confirmation. Currently, available datasets do not provide such detailed localization.**

**To address this limitation, our analysis was based on the working hypothesis that the CA1 region generates distinct sharp-wave ripples (SWRs), supported by substantial research evidence. Consequently, our study focused on comparing our findings from the presumed CA1 regions not with other hippocampal subregions, but rather with results from the amygdala and the entorhinal cortex, as shown in Figures 2C–E.   
To enhance clarity in our manuscript, we have revised our description as follows:**

**Our analysis focused on putative CA1 regions (Figure 4) in order to en-**

**hance the validity of recording site and the true positive rate of SWR de-**

**tection. This criterion is supported by accumulated evidence. For instance,**

**SWRs synchronize with spike bursts of interneuron and pyramidal neuron**

**[54, 55, 56, 57], potentially within a 50 µm radius of the recording site [58].**

**Additionally, we identified increased incidence of SWRs during the first 0–**

**400 ms of the retrieval phase (Figure 4D). This finding aligns with previ-**

**ous reports of heightened SWR occurrence preceding spontaneous verbal re-**

**call [21, 22], extending our understanding to a triggered retrieval condition.**

**Moreover, the log-normal distributions of both SWR duration and ripple**

**band peak amplitude observed in this study (Figure 4C & E) coincide with**

**the consensus in this field [49]. Therefore, these results support the electrode**

**placement and detected SWRs in this study. One could argue that the neural**

**trajectory (NT) distance increase from O during sharp wave-ripples (SWRs)**

**(Figure 5) may be artificially inflated towards higher values due to channel**

**selection using UMAP clustering on spike counts. However, this potential**

**bias does not affect the direction of NT, the memory-load dependency, nor**

**the WM task dependency identified in this study.**

### **# Reviewer 1’s Comments 6 - 1:**

*In the discussion section, the authors discovered a positive correlation between set size and the neural trajectory (NT) distance during the encoding and retrieval phases, not for other phase combinations. An explanation for this phenomenon would be beneficial.*

**Authors’ Response:**

**Actually, we found interesting results for other phases. However, since the combination of phases will explode the focus and make the readability lower, we focused on encoding and retrieval phases.**

**The dataset is publicly available about two years ago. After that, various researchers conducted using the dataset. However, we are the first to focus on sharp-wave ripples. Prior findings shows the relation and contribution of SWRs primarily in long-term memory context, but in contrast, we extended insights for WM.**

### **calc**

### **# Reviewer 1’s Comments 6 - 2:**

*Besides, has similar analyses done in previous studies of working memory or cognitive functions?*

**Authors’ Response:**

**Thank you for inquiring about whether similar analyses have been conducted in previous studies of working memory or cognitive functions.**

**While numerous researchers have utilized this publicly available dataset over the past two years, our analysis uniquely focuses on the role of sharp-wave ripples (SWRs) within specific memory phases. Previous studies leveraging this dataset have primarily not addressed SWRs, especially not within the context of working memory tasks.**

**Our investigation stands out because it specifically examines the impact of SWRs during the encoding and retrieval phases, which are critical for understanding the dynamics of working memory. Most prior research involving SWRs has explored their contributions mainly within the context of long-term memory. By extending the investigation to working memory, our study provides novel insights that bridge a significant gap in the existing literature.**

**This focus allows us to uncover unique aspects of how SWRs may influence working memory processes, offering new perspectives on their functional roles in cognitive tasks that have not been previously detailed. Hence, our study not only contributes to the understanding of SWRs in a new context but also enhances the broader field of memory research by introducing fresh methodologies and findings to the discourse on cognitive functions.**

**To incorporate these backgrounds, we have updated the Introduction section as follows:**

### **Lines XX–XX:**

**…**

### **# Reviewer 1’s Comments 6 - 3:**

*How do the discoveries of this research contrast with prior findings?*

**Authors’ Response:**

**Thank you for your question about how our findings contrast with prior research.**

**Our study provides fresh insights into the role of sharp-wave ripples (SWRs) in working memory (WM), particularly during the encoding and retrieval phases. Traditionally, research on SWRs has primarily focused on their involvement in long-term memory, especially during periods of rest or sleep, with an emphasis on their role in memory consolidation and retrieval. Additionally, prior studies exploring SWRs in the context of working memory often suffered from low temporal resolution, which hindered precise identification of the specific timings for memory encoding and retrieval.**

**In contrast, our research utilized a high temporal resolution dataset, an approach not commonly employed in previous SWR studies. This method allowed us to capture the dynamics of SWRs with exceptional precision during active cognitive tasks. As a result, we identified a state-switching role for SWRs, indicating their active involvement in real-time memory processing tasks.**

**These findings challenge the traditional views that link SWRs primarily to long-term memory processes and suggest a broader, more dynamic role for SWRs in cognitive functions. By demonstrating that SWRs are integral not only to the consolidation of long-term memories but also to the active processing phases of working memory, our research broadens the known functional scope of SWRs, potentially influencing future investigations into their role across different types of memory tasks.**

**To incorporate these backgrounds, we have updated the Introduction section as follows:**

### **Lines XX–XX:**

**…**

### **# Reviewer 1’s Comments 7:**

*There are some typoes, please fix them, for example:*

*1)on page 2, line 94, (Figure ??S1)*

*2)on page 4, lines 219-220, (Figures 3D and ??)*

**Authors’ Response:**

**Thank you very much. We missed the addition of supplementally figures. In this revision, we have included them and confirmed that referenced figures are linked correctly.**

### **supplementary**

## **Reviewer 2:**

### **Reviewer 2’s Comments 1:**

*Reviewer 2: 1. The authors provide sufficient details for most of the analysis. However, some methods are presented with insufficient details.*

*2. All the results that support the conclusion are directly shown.*

*3. I find that the conclusion drawn by the authors are an exaggerated extension of the results obtained. I think a major flaw is present in the definition of 'states', which are the core of the study. Clear definition of the term 'state' is needed, together with additional analysis to show their existence. At this stage, the results are too weak to support the conclusion.*

*4. The study complies with the ethical guidelines.*

**Authors’ Response:**

**We appreciate your constructive feedback.**

### **# Reviewer 2’s Comments 2:**

*Major comments:*

*- Defining 'encoding' and 'retrieval' states requires a careful, complete, and clear analysis. From Fig.2, it seems that NT points are scattered and do not have stable 'states'. This could be a major flaw of the study, since the 'transition between states' could be no longer valid. To define states, authors would need to explicitly show that points that belong to 'encoding' and 'retrieval' phases are clustered in different regions of the state space and have small overlap.*

**Authors’ Reponse:**

### **calc**

### **# Reviewer 2’s Comments 3:**

*- The results presented in Fig.7 needs to be compared with the cosine distance distribution of random vectors in the 3-dimensional space. Among the ones shown in Fig.7, which are the significant results?*

**Authors’ Response:**

### **calc**

### **# Reviewer 2’s Comments 4:**

*- From Fig.1, the encoding NT seems to be close to 0 the whole time, apart from when SWR+ appears. To validate this switching between encoding and retrieval states, the authors would need to compare the cosine similarity distribution of rSWR+ with gEgR, and the distribution of rSWR+ with gR. If the authors find no significant difference in the two distributions, then they cannot claim the switching between encoding and retrieval states.*

**Authors’ Response:**

### **calc**

### **Reviewer 2’s Comments 5:**

*- The authors do not provide sufficient details on the SWR clustering with UMAP. Please define how you define the silhouette score and which features do you use for clustering.*

**Authors’ Response:**

**Thank you very much. We have updated the corresponding method section with modifications to reduce ambiguity.**

**2.4. Defining SWRs from Putative Hippocampal CA1 Regions Using UMAP**

**Clustering**

**Potential SWRs were differentiated from SWR candidates in putative**

**CA1 (cornu Ammonis 1) regions. The definition of putative CA1 regions**

**was as follows. First, SWR + and SWR − candidates in the hippocampus were**

**projected into a two-dimensional space using a supervised clustering method,**

**Uniform Manifold Approximation and Projection (UMAP) [51]. The input**

**features for this projection were the spike counts per unit during the period**

**of SW R + or SW R − candidates. Clustering validation was performed by**

**calculating the silhouette score [52] from clustered sample points in the cor-**

**responding two-dimensional space. Regions in the hippocampus that scored**

**above 0.6 on average across sessions (75 th percentile) were identified as puta-**

**tive CA1 regions. This process resulted in the identification of five electrode**

**positions from five patients.**

**SWR + /SWR − candidates in these predetermined CA1 regions were cat-**

**egorized as SWR + /SWR − , and thus they no longer retained their candidate**

**status. The duration and ripple band peak amplitude of SWRs were found**

**to follow log-normal distributions. Each time period of SWR was partitioned**

**relative to the time from the SWR center into pre- (at −800 ms to −300 ms**

**from the SWR center), mid- (at −250 to +250 ms), and post-SWR (at +300**

**to +800 ms) times.**

### **Reviewer 2’s Comments 6:**

*- I find that section 3.6 and Fig.6 are not adding any information to the study. The results obtained with the initial 3-dimensional projection provide the same results as the ones in section 3.6.*

**Authors’ Response:**

**Thank you very much. We moved Section 3.6 and Fig. 6 to the supplementary file.**

### **Reviewer 2’s Comments 7:**

*- The authors find positive correlation between set size and the distance between median NT position in encoding (gE) and median NT position in recall (gR). Distances are log-transformed. Is this correlation present also when distances are not log-transformed? I find that the reason presented in Discussion for log- transformation ('log-normal distributions are prevalent in the central nervous system') is not valid. I think that this Discussion paragraph should be deleted.*

**Authors’ Response:**

**Thank you very much for your suggestion. Correlations were not found when log-transformation is not applied. However, pair-wise comparisons remains the same statistics as we used Brunner-Munzel test, which is an non-parametric method based on rank data.**

**Following your suggestion, we have deleted the discussion paragraph.**

### **# Reviewer 2’s Comments 8:**

*Minor comments:*

*- Is the result shown in Fig.2c from a single patient?*

**# Authors’ Response:**

**# Check the data**

### **Reviewer 2’s Comments 9:**

*- Fig.3c is not informative. By definition, SWR+ and SWR- will have the same duration distribution.*

**Authors’ Response:**

**We believe you are referring to Fig. 4c, not Fig. 3c. The purpose of Fig. 4c is not solely to display the identical distribution of SWR+ and SWR-, but also to serve as supporting evidence for the detection of SWR events, which ideally follow a log-normal distribution. To clarify these points, we have updated the legend of Fig. 4c.**

**Figure 4 – Detection of SWRs in Putative CA1 Regions**

**A. Two-dimensional UMAP [51] projection displays multi-unit spikes during**

**SWR + candidates (purple) and SWR − candidates (yellow ). B. A cumulative**

**density plot indicates silhouette scores, reflecting UMAP clustering quality (see**

**Table 2). Hippocampal regions with silhouette scores exceeding 0.60 (equiv-**

**alent to the 75 th percentile) are identified as putative CA1 regions. SWR +**

**and SWR − candidates, which were recorded from these regions, are classified**

**as SWR + and SWR − respectively (ns = 1,170). C. Identical distributions**

**of SWR + (purple) and SWR − (yellow ) distributions, based on their defini-**

**tions (93.0 [65.4] ms, median [IQR]). Note that these distributions exhibit log-**

**normality. D. Identical SWR incidence for both SWR + (purple) and SWR −**

**(yellow ), relative to the probe’s timing (mean ±95% confidence interval). How-**

**ever, 95% confidence interval may not be visibly apparent due to their narrow**

**ranges. Note that a significant SWR incidence increase was detected during**

**the initial 400 ms of the retrieval phase (0.421 [Hz], \*p < 0.05, bootstrap test).**

**E. Distributions of ripple band peak amplitudes for SWR − (yellow ; 2.37 [0.33]**

**SD of baseline, median [IQR]) and SWR + (purple; 3.05 [0.85] SD of baseline,**

**median [IQR]) are manifested (\*\*\*p < 0.001, the Brunner–Munzel test). Note**

**the log-normality for SWR + events.**

### **Reviewer 2’s Comments 10:**

*- Contrary to what is written in the Figure caption, Fig.4d shows the mean SWR incidence, but not the confidence interval. In addition, the SWR- incidence is not present.*

**Authors’ Response:**

**Thank you very much for your query. We have updated the legend of Fig. 4d.**

**Figure 4 – Detection of SWRs in Putative CA1 Regions**

**A. Two-dimensional UMAP [51] projection displays multi-unit spikes during**

**SWR + candidates (purple) and SWR − candidates (yellow ). B. A cumulative**

**density plot indicates silhouette scores, reflecting UMAP clustering quality (see**

**Table 2). Hippocampal regions with silhouette scores exceeding 0.60 (equiv-**

**alent to the 75 th percentile) are identified as putative CA1 regions. SWR +**

**and SWR − candidates, which were recorded from these regions, are classified**

**as SWR + and SWR − respectively (ns = 1,170). C. Identical distributions**

**of SWR + (purple) and SWR − (yellow ) distributions, based on their defini-**

**tions (93.0 [65.4] ms, median [IQR]). Note that these distributions exhibit log-**

**normality. D. Identical SWR incidence for both SWR + (purple) and SWR −**

**(yellow ), relative to the probe’s timing (mean ±95% confidence interval). How-**

**ever, 95% confidence interval may not be visibly apparent due to their narrow**

**ranges. Note that a significant SWR incidence increase was detected during**

**the initial 400 ms of the retrieval phase (0.421 [Hz], \*p < 0.05, bootstrap test).**

**E. Distributions of ripple band peak amplitudes for SWR − (yellow ; 2.37 [0.33]**

**SD of baseline, median [IQR]) and SWR + (purple; 3.05 [0.85] SD of baseline,**

**median [IQR]) are manifested (\*\*\*p < 0.001, the Brunner–Munzel test). Note**

**the log-normality for SWR + events.**

### **Reviewer 2’s Comments 11:**

*- In Fig.3 the authors do not use SWR information. The reference to Fig.3 in section 3.6 is not correct.*

**Authors’ Response:**

**We applogie for the confusion. We intended to reiterate our findings to show the motivation to focus on the encoding and retrieval phases, because we found memory-load dependent NT distances between these phases.**

**Upon considering, we have decided to delete the text as follows:**

**Having observed NT ’jumping’ during SWR (Figure 5), we visualized the three-dimensional NTs of pre-, mid-, and post-SWR events during the encoding and retrieval phases (Figure 6).**

**To provide two-dimensional visualization, we linearly …**

### **Reviewer 2’s Comments 12:**

*- In Discussion, the phrase 'hippocampal neurons form unique NTs, primarily during SWR' is not correct. Hippocampal activity always forms NT, by definition. Please remove or rephrase.*

**Authors’ Response:**

**Thank you very much. We have revised the sentence as follows:**

**This study hypothesizes that in low-dimensional spaces during a WM task in humans, hippocampal neurons exhibit WM-task dependent NTs, primarily during SWR periods.**

### **Reviewer 2’s Comments 13:**

*- In Discussion, the main findings (distance of NT bigger in hippocampus, correlation between set size and gEgR) are repeated twice.*

**Authors’ Response:**

**Thank you very much for your suggestions. We now separated the results and implications in different paragraphs, probably the cause of the redundancy. We hope these amendments have solved the repetitions.**

**4. Discussion**

**This study hypothesizes that in low-dimensional spaces during a WM task**

**in humans, hippocampal neurons form unique NTs, primarily during SWR**

**periods. Initially, multi-unit spikes in the MTL regions were projected onto**

**three-dimensional spaces during a Sternberg task using GPFA (Figure 1D–**

**E & Figure 2A). The NT distances across WM phases (kg F g E k, kg F g M k,**

**kg F g R k, kg E g M k, kg E g R k, and kg M g R k) were significantly larger in the hip-**

**pocampus compared to the EC and amygdala (Figure 2C–E). Also, in the**

**hippocampus, the NT distance between the encoding and retrieval phases**

**(kg F g E k) positively correlated with memory load (Figure 3C–D). The hip-**

**pocampal NT transiently expanded during SWRs (Figure 5). Lastly, the**

**hippocampal NT alternated between encoding and retrieval states, transi-**

**tioning from encoding to retrieval during SWR events (Figure 7). These**

**findings explain aspects of hippocampal neural activity during a WM task**

**in humans and offer new insights into SWRs as a state-switching element in**

**hippocampal neural states.**

**The longer disntace of NTs across the four phases in the hippocampus**

**indicates dynamic and responsive neural activity in the hippocampus dur-**

**ing the WM task. This observation corroborates previous studies indicat-**

**ing hippocampal persistent firing during the maintenance phase [4, 5, 6, 3].**

**However, in the present study, applying GPFA to multi-unit activity dur-**

**ing a one-second level resolution of the WM task revealed that the NT in**

**low-dimensional space presented a memory-load dependency between the en-**

**coding and retrieval phases, denoted as kg E g R k (Figure 3). These findings**

**support the association of the hippocampus with WM processing.**

### **Reviewer 2’s Comments 14:**

*- In Discussion, the phrase 'the potential bias does not substantially challenge our main findings' is not clear. Why does the potential bias do not affect the main findings?*

**Authors’ Response:**

**Thank you very much. We have explained it in details, which is necessary for communication with readers.**

**One could argue that the NT distance increase from O during SWRs (Figure 5) may be artificially inflated towards higher values due to channel selection using UMAP clustering on spike counts. However, this potential bias does not affect the direction of NT, the memory-load dependency, nor the WM task dependency identified in this study.**

*\*\*\*\*\**

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# **Conclusion**

We extend our gratitude to the Editor and Reviewers for their insightful comments and suggestions, which have significantly enhanced the quality of our manuscript. We have diligently addressed the concerns raised during the review process and hope that the revised manuscript will meet the publication standards of ***Heliyon***.