

Peer Review File

Coordinated NREM Sleep Oscillations among Hippocampal Subfields Modulate Synaptic Plasticity in Humans



Open Access This file is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. In the cases where the authors are anonymous, such as is the case for the reports of anonymous peer reviewers, author attribution should be to 'Anonymous Referee' followed by a clear attribution to the source work. The images or other third party material in this file are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The manuscript does not have page or line numbers which complicates the review.

The manuscript compares 3 hippocampal subfields in terms of oscillatory phenomena, which is of interest as it provides additional information on how neural processing occurs during NREM sleep.

The analysis is based on aggregate data from a number of subjects; it would be important to add information related to single subjects in a revision. Major aspects of the analysis should document variability between subjects.

The manuscript is not hypothesis driven; the authors performed an exhaustive set of analyses which is linked to some previous findings in the literature during the discussion. A more directed, hypothesis-driven, approach would be much preferable, with close focus on the main important and specific findings.

Please find below some more detailed comments on the manuscript.

Results

NREM epochs were selected as showing prolonged elevations in the low delta band, reduced high gamma and elevated spindle activity. Were all of these criteria applied in the selection procedure? Which hippocampal subfield was analyzed for this selection; did all subfields have to show the 3 criteria to pass threshold? Please report mean and std err of NREM episode length across subjects.

It is stated as a result that high delta and spindle frequency power were observed in NREM sleep, but these are the criteria used for selection! Reporting effects for which sites were selected is circular. Please explain clearly the logic of data analysis while avoiding such confounds.

The inset of Fig1b is unreadable. What exactly is a “cluster correction”? The authors seem to have employed a multitude of t-tests, where great care must be taken to correct for multiple comparisons. The data as presented appear a little anecdotal. Please show main effects as scatter plots including all subjects, so the between subject variability can be

examined.

Fig.2b: there is no apparent enhancement in theta range for DG/CA3 vs. SUB, yet the statistical analysis reveals this. Please explain. Showing data for single subjects would be desirable also here.

Fig.3: There is no systematic relationship between coupling phase in the three subfields. Please explain in more detail how these significant coupling phases are computed. The findings suggest different mechanisms are present in the three subfields for these couplings which seems unlikely.

Discussion:

The authors refer to modulations in synaptic plasticity modulations; yet these were not directly measured; please specify how this inference is made.

The authors contrast SO (slow oscillations 0.2-1.7Hz) with delta waves (1-4Hz); these phenomena are partially overlapping in frequency, so it is not possible to separate their functions. Such a dissociation would necessitate non-overlapping frequencies and computing coupling of ripples, etc to both of the candidate low frequency modulations and seeing which one is more pronounced. This would be a worthwhile enterprise and would enhance the usefulness of the present study.

Reviewer #2 (Remarks to the Author):

Li et al. present a study on oscillatory activity in the hippocampus during NREM sleep with a focus on the synaptic plasticity. A strength of the study is the relatively large sample and that the authors use intracranial recordings. The authors record from the CA3, CA1 and SUB regions. The authors report for a strong coupling between delta band activity in ripples in the places (especially DG/CA3). Especially in the CA1 and SUB fields there was strong coordination between activities. The study is of interest given the role memory consolidation processes in NREM sleep. The introduction is very balanced and in-depth and the study question is clear and relevant. The placement of the electrodes seems accurate and was based on the MR-navigation during surgery. From a methodological point of view, the study also seems sound (i.e. PAC was utilized using the appropriate implementation). The authors perform a multi-level analysis of spindle-ripple couplings

and this line-up of these analyses is well-motivated. It is easy to follow the results section and the figures are clear. The same goes for the discussion section. My only minor point here is that the authors present the discussion in sections making it a bit difficult to fully comprehend the overarching picture of the study and the advance presented. I ask the authors to provide another structure in the discussion.

1 Reviewer #1:

2 Q1: The manuscript does not have page or line numbers which complicates the review.

3 Response: We apologize for this oversight. Page and line numbers have now been included in
4 the revised manuscript to facilitate easier navigation and referencing during the review process.

5 Q2: The analysis is based on aggregate data from a number of subjects; it would be important
6 to add information related to single subjects in a revision. Major aspects of the analysis should
7 document variability between subjects.

8 Response: Thank you for bringing this issue to our attention. In response to the specific
9 questions posed (Q6 and Q7), we have included additional information about individual
10 subjects for our main results.

11 Q3: The manuscript is not hypothesis driven; the authors performed an exhaustive set of
12 analyses which is linked to some previous findings in the literature during the discussion. A
13 more directed, hypothesis-driven, approach would be much preferable, with close focus on the
14 main important and specific findings.

15 Response: Thank you for your insightful feedback on our manuscript. We appreciate your
16 observation that our study appears to lack a hypothesis-driven approach, and we agree that it
17 would be beneficial to focus more closely on specific research findings. While we performed
18 an exhaustive set of analyses to consider various potential factors influencing our study, we
19 acknowledge that a hypothesis-driven approach would have been more precise. Thus, we have
20 made thorough revisions to our manuscript, particularly in the introduction, results, and
21 discussion sections. These revisions result in a greater emphasis on our primary findings,
22 focusing on the intricate coupling of delta, spindle and ripple oscillations among the
23 hippocampal subfields and the distinct modulation of synaptic changes by hippocampal
24 spindles and ripples during NREM sleep.

25 In the introduction section, we have revised the presentation of our scientific questions. In
26 contrast to the original manuscript, we now explicitly state two main hypotheses as follows:

27 *In lines 87 to 95 on page 2:*

28 *“Given that oscillatory coupling has been established as a critical mechanism for interregional*
29 *information transmission in the brain^{18, 32, 33}, we hypothesize that information coordination*
30 *within the hippocampal circuit can be achieved through tight coupling among the cardinal*
31 *NREM oscillations between different hippocampal subfields. Moreover, increasing evidence*
32 *has suggested that each hippocampal subfield plays a unique role in processing information*
33 *essential for memory formation and consolidation^{26, 34-37}. Thus, we hypothesize further that*
34 *different hippocampal subfields may exhibit distinct profiles of sleep oscillation coupling*
35 *during NREM sleep.”*

36 *In line 108 to 116 on page 3:*

37 *“While the delta wave activity has been considered an indirect but effective marker of synaptic*
38 *plasticity and homeostasis, the other sleep events including spindle and ripple have been*
39 *suggested to provide crucial time windows for synaptic reshaping. However, there is a lack of*

research investigating the specific impact of hippocampal spindle and ripple oscillations on synaptic potentiation during NREM sleep. We thus hypothesize that hippocampal spindles and ripples may have distinct roles in regulating synaptic potentiation, which could be reflected in their associations with changes in delta-wave slope during NREM sleep.”

By directly pinpointing the core issues, we aim to clarify the focus of our study and enhance the clarity of our conclusions.

In the results section, to focus the manuscript more on our primary findings, we have removed the part titled “Weak delta-band synchrony within hippocampus during NREM”, as it did not closely align with our main discoveries. Moreover, we have restructured the discussion section to improve its coherence, making it more accessible and emphasizing our study’s pivotal discoveries.

Q4: NREM epochs were selected as showing prolonged elevations in the low delta band, reduced high gamma and elevated spindle activity. Were all of these criteria applied in the selection procedure? Which hippocampal subfield was analyzed for this selection; did all subfields have to show the 3 criteria to pass threshold? Please report mean and std err of NREM episode length across subjects.

Response: We apologize for the lack of detail in explaining the selection process for NREM epochs. To select NREM epochs, we followed the methodology used in previous studies (Jiang et al., 2017, 2020), which utilized SEEG data for NREM detection. In line with Jiang et al., we followed three criteria during the selection process: prolonged elevations in the low delta band, reduced high gamma activity, and elevated spindle activity. All the three criteria should be met when selecting NREM epochs.

Furthermore, since these criteria are primarily observed in neocortical regions, we focused on electrodes located in the neocortex rather than those in the hippocampus when selecting NREM epochs. As the number and placement of SEEG electrodes differ among participants, the specific neocortical electrodes used for NREM detection varied between individuals. However, to ensure the validity and accuracy of our NREM detection, for each participant, we only selected epochs in which the vast majority of the neocortical electrodes met all three criteria.

Additionally, we further ensured the accuracy of the selected NREM epochs by manually reviewing the SEEG waveforms and retaining only the epochs for which we had high confidence in our NREM sleep determination. As a result, the NREM epochs included in our analysis are relatively shorter in duration but were chosen with a focus on maintaining a high level of confidence in their accuracy.

In the revised manuscript, we have included these details in the relevant sections (please see lines 130 to 135 on page 5, and lines 498 to 450 on page 29). We have also provided the mean and standard error of NREM episode length across subjects in Supplementary Table 1 of the manuscript.

In the revised manuscript:

“Due to the lack of clinical sleep hypnograms, patient monitoring videos, scalp EEG, and electrooculograms, we selected NREM sleep (including N2 and N3 stages) based on neocortical

iEEG signals by identifying prolonged elevations in low delta band (<2 Hz) activity amplitude and corresponding declines in high-gamma (HG; 70–190 Hz) and initially high but gradually decreasing spindle band amplitudes (Fig. 1a).”

“When selecting NREM epochs for sleep staging, we focused solely on the electrodes located in the neocortex. NREM sleep was identified only if the majority of these neocortical electrodes met the aforementioned criteria.”

SUBJECT	DURATION OF RECORDING [H]	NUMBER OF NREM EPOCHS	DURATION OF NREM [MIN]	AVERAGE NREM DURATION PER EPOCH [MIN]
1	28	6	102	17
2	6.1	3	75	25
3	31.8	4	60	15
4	3.7	4	63	15
5	6	5	116	23
6	24	3	84	28
7	39.2	6	153	25
8	16	2	60	30
9	30	4	60	15
10	32	2	33	16
11	48	4	167	41
12	7.7	5	96	19
13	5.8	6	201	33
14	48	6	171	28
15	5.8	5	39	7
16	6.2	3	63	21
17	29.5	2	30	15
18	2	2	30	15
19	8.3	6	158	26
20	21.4	4	111	27
21	23.9	4	84	21
22	6.1	2	54	27
23	32	2	39	19
24	39	2	45	22
25	39.2	3	51	17
GROUP AVERAGE (MEAN ± SD)	21.588 ± 14.7	3.8 ± 1.5	85.8 ± 48.7	21.88 ± 7.2

Supplementary Table 1 Sleep metrics of each subject. Participants underwent continuous recording for several days, from which we selected several nights of sleep. Due to the

conservative approach, the extracted NREM (including N2 and N3, but excluding N1) sleep duration was relatively shorter.

References:

Jiang, X. et al. Replay of large-scale spatio-temporal patterns from waking during subsequent NREM sleep in human cortex. *Sci. Rep.* 7, 17380 (2017).

Jiang, X. et al. Improved identification and differentiation from epileptiform activity of human hippocampal sharp wave ripples during NREM sleep. *Hippocampus* 30, 610-622 (2020).

Q5: It is stated as a result that high delta and spindle frequency power were observed in NREM sleep, but these are the criteria used for selection! Reporting effects for which sites were selected is circular. Please explain clearly the logic of data analysis while avoiding such confounds.

Response: Thank you for your comment. We understand that it can lead to confusion as our previous manuscript didn't mention clearly that only neocortical electrodes are used for NREM sleep identification. We have included the details of NREM sleep selection in the revised manuscript (also see our response to Q4), which should make it clear that only neocortical electrodes were used for this process.

To further clarify, while we ensured high delta and spindle activity in the selection of NREM sleep, the electrodes used were from the neocortex rather than the hippocampus. Thus, the spectral properties of the hippocampus in NREM sleep still requires further investigation.

Furthermore, in this section, we also compared the spectral differences among hippocampal subfields to investigate if different subfields displayed different spectral profiles. We analyzed the spectral power of different frequency bands in different hippocampal subfields and found significant differences between certain subfields. Specifically, we found that the DG/CA3 showed an overall trend of higher power than the CA1, and SUB, and significant in 10–11 Hz and 27–30 Hz; But after normalization, the spindle band of SUB was significantly higher than that of DG/CA3. This analysis provided insights into the heterogeneity of hippocampal activity during NREM sleep. To more accurately reflect our focus, we have modified the title of this section in the revised manuscript to “Spectral profiles of different hippocampal subfields during NREM sleep”. We hope these clarifications help to better convey the findings of the section.

Our modification in the results section is line 127 on page 5.

In the revised manuscript:

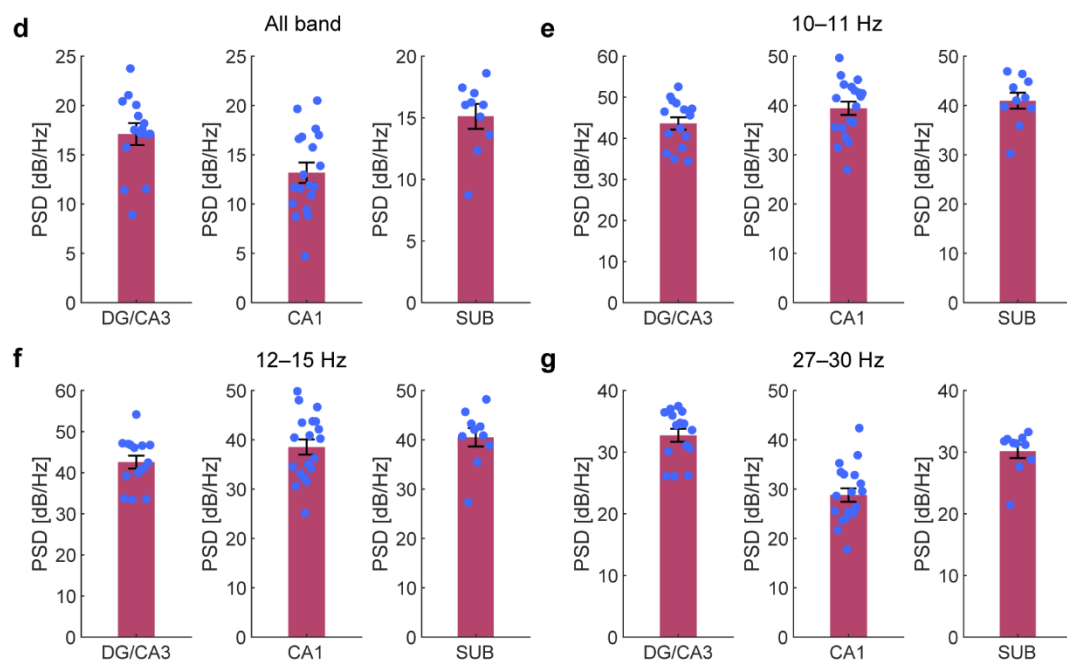
“Spectral profiles of different hippocampal subfields during NREM sleep”

Q6: The inset of Fig1. b is unreadable. What exactly is a “cluster correction”? The authors seem to have employed a multitude of *t*-tests, where great care must be taken to correct for multiple comparisons. The data as presented appear a little anecdotal. Please show main effects as scatter plots including all subjects, so the between subject variability can be examined.

Response: Thank you for your comments. We apologize for the imprecise wording used to describe the “cluster correction” method. Our intention was to refer to the commonly employed

cluster-based permutation correction method in the analysis of EEG/MEG/SEEG data. We have revised the manuscript accordingly, replacing the term “cluster correction” with “cluster-based permutation test”. Because MEG/EEG/iEEG data usually involves a substantial number of channel/time/frequency-pair comparisons, it is impossible to use traditional multiple comparison methods for correction. To address this issue, the cluster-based permutation test has been suggested as a solution. More information about the specific principles of this method can be found on the FieldTrip official website at https://www.fieldtriptoolbox.org/tutorial/cluster_permutation_timelock/.

To improve the presentation of our data, we have added scatter plots displaying the spectral results from all individual subjects to Supplementary Fig. 2d–g. These scatter plots provide a more comprehensive view of the data at an individual level. As can be seen from these figures, there are no substantial individual variations in the power of each band.



Supplementary Fig. 2d Scatter plots of the spectral mean in the three hippocampal subfields for the frequency range of 0–200 Hz. The bar and error bar plots represent the mean and SEM across all subjects, and blue dots represent individual subjects. **e–g** Similar to **d**, scatter plots of spectral means in three specific frequency bands (10–11 Hz, 12–15 Hz, and 27–30 Hz), which exhibit significant differences between hippocampal subfields.

Q7: Fig. 2b: there is no apparent enhancement in theta range for DG/CA3 vs. SUB, yet the statistical analysis reveals this. Please explain. Showing data for single subjects would be desirable also here.

Response: Thank you for bringing this to our attention. Upon re-examining the data, we have observed relatively high individual variability in phase-amplitude coupling values for the SUB region (see figure below), which may account for why there is “no apparent enhancement in the theta range, despite statistical analysis revealing differences”. Therefore, in the revised manuscript, we have removed the analysis comparing PAC values between hippocampal subfields to prevent inaccurate statistical results.

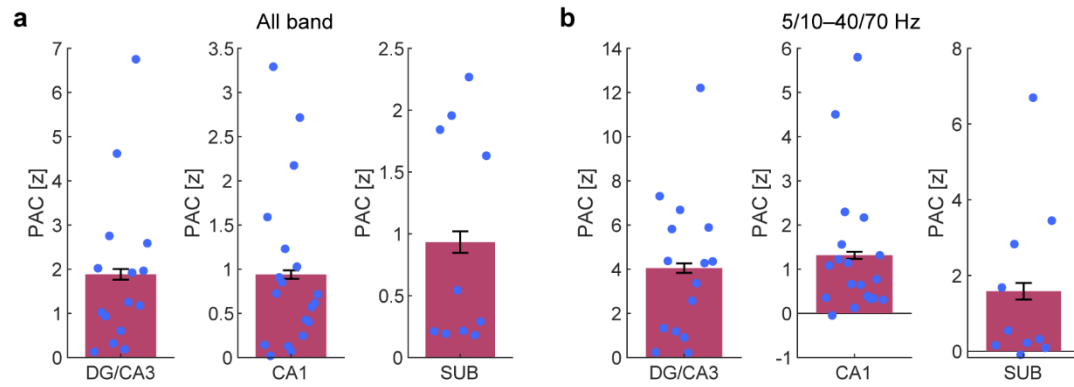


Figure 1. Scatter plots of the phase-amplitude coupling (PAC) averaged across (a) all frequency bands and (b) theta band (5/10–40/70 Hz) of three hippocampal subfields. The bars and error bars represent the mean and SEM across subjects, while the blue dots indicate individual subjects.

Q8: Fig.3: There is no systematic relationship between coupling phase in the three subfields. Please explain in more detail how these significant coupling phases are computed. The findings suggest different mechanisms are present in the three subfields for these couplings which seems unlikely.

Response: Thank you for your valuable comments. We estimated the significance of coupling phases among delta/SO, spindle and ripple oscillations according to previous research (Staresina et al., 2015). Specifically, as detailed in the methods section, we first filtered the raw signals for the frequencies of interest (SO: 0.2–1 Hz; delta: 1–4 Hz; spindle: 12–16 Hz; ripple: 80–100 Hz) within the spindle event epoch. We then used the Hilbert transform to extract the phase values of the lower-frequency oscillation (modulating) and the phase values of the power fluctuations of the higher-frequency oscillation (modulated). Next, we computed the coupling phase by evaluating the angle of synchronization index (SI; a complex number) between pairs of phase time series (modulating and modulated) for each event epoch. For the statistical analysis of phase angles, the V test, which assesses the non-uniformity of circular data with a specified mean direction, serves as a robust alternative to the Rayleigh test (Berens et al., 2009). For the statistical analysis of phase angles, we used the V test, which serves as a powerful alternative to the Rayleigh test, to test for non-uniformity of circular data with a specified mean direction (Berens et al., 2009). We pooled the resulting phase values across all participants and assessed whether certain phase values were significantly overrepresented compared to a uniform distribution, followed by Bonferroni multiple comparison correction.

We appreciate your comment regarding the possible presence of different mechanisms for the couplings in different hippocampal subfields. We would like to clarify that while the DG/CA3, CA1, and SUB all belong to the hippocampal formation, it does not necessarily imply that they cannot have different functions or processing mechanisms. In fact, emerging evidence has suggested that different hippocampal subfields may have distinct functions and processing modes. We would like to highlight some of these evidences. Firstly, the projections among the hippocampal subfields (DG, CA3, CA1, and SUB) form intricate and highly organized circuits. For example, the DG receives inputs from EC and projects to CA3, while CA1 receives inputs

from both EC and CA3 and projects to SUB as well as back to EC. These distinct projection patterns among the hippocampal subfields may underlie their different processing modes and roles in memory formation and consolidation. Secondly, prior research has shown that different hippocampal subfields play different roles in learning and memory processes (Gilbert et al., 2001; Lee & Kesner, 2002; Lee et al., 2004; Lasztóczy & Klausberger, 2017; Meier et al., 2020). For instance, the DG has been shown to have a pattern separation role while CA3/CA1 subfields are involved in pattern completion (Mizumori et al., 1989; Bakker et al., 2008). Moreover, previous studies have found a dissociation of the function in DG/CA3 and CA1 in terms of the NMDA receptor and backward shift in place fields in hippocampal-dependent memory task (Lee & Kesner, 2002; Lee et al., 2004), suggesting that DG/CA3 plays a key role in the rapid formation of representations of new information, whereas CA1 may be more important for comparing current experience with stored representations in the CA3 network. Furthermore, different neural oscillatory patterns, including frequency distribution, temporal organization and cross-frequency coupling have been found in DG/CA3 and CA1 areas (Lasztóczy & Klausberger, 2017; Meier et al., 2020), suggesting that network operations could be segregated in different hippocampal subfields. Based on all the available evidence, we believe that it is reasonable to postulate the existence of different mechanisms for different hippocampal subfields, which can be reflected by their distinct rhythmic synchronization patterns.

Our results align with these previously identified functional distinctions between hippocampal subfields. Specifically, our results indicate extensive coupling in both time and space within DG/CA3, while CA1 exhibits the strongest delta-spindle-ripple event-based coupling compared to DG/CA3 and SUB. The key distinction between event-based coupling and band-based phase-amplitude coupling lies in timescale: event-based coupling reflects short-term coupling strength associated with specific events, whereas band-based phase-amplitude coupling represents an average over longer timescales. Thus, these different coupling patterns in the three hippocampal subfields indicate their dissociable roles in information routing and processing, where the DG/CA3 may process information in a more global manner, while the CA1 may handle more precise information processing that relies on intricate modulations to integrate current cortical input with previously stored representations in the DG/CA3. Overall, our findings offer further support for the idea that different hippocampal subfields have distinct functions and processing modes. In the revised manuscript, we have expanded our discussion to offer a more comprehensive explanation regarding the potential dissociation in the roles of hippocampal subfields, as evidenced by their distinct rhythm coupling patterns. Please see lines 287 on page 15 to line 342 on page 16.

References:

- Staresina, B. P. et al. Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat. Neurosci.* 18, 1679-1686 (2015).
- Berens, P. et al. CircStat: a Matlab Toolbox for Circular Statistics. *Journal of Statistical Software.* 31, 1-21 (2009).
- Gilbert, P. E. et al. Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. *Hippocampus.* 6, 626-636 (2001).

Lee, I. & Kesner, R. P. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. *Nat Neurosci.* 5, 162-8 (2002).

Lee, I. et al. A double dissociation between hippocampal subfields: differential time course of CA3 and CA1 place cells for processing changed environments. *Neuron.* 42, 803-15 (2004).

Lasztóczy, B. & Klausberger, T. Distinct gamma oscillations in the distal dendritic fields of the dentate gyrus and the CA1 area of mouse hippocampus. *Brain Struct Funct.* 222, 3355-3365 (2017).

Meier K, et al. Dentate Gyrus Sharp Waves, a Local Field Potential Correlate of Learning in the Dentate Gyrus of Mice. *J Neurosci.* 40, 7105-7118 (2020).

Q9: The authors refer to modulations in synaptic plasticity modulations; yet these were not directly measured; please specify how this inference is made.

Response: Thank you for your valuable comment. While we did not directly measure synaptic plasticity in our study, our approach involved monitoring changes in delta wave slope across NREM sleep. A steeper delta wave slope is indicative of stronger and more numerous synaptic connections. Conversely, a reduction in this slope during sleep suggests synaptic downscaling, where synaptic strength and density are pruned to maintain homeostasis. This method is widely accepted in the field as an indirect but effective measure of synaptic plasticity and homeostasis (Tononi & Cirelli, 2006; Kurth et al., 2010; Knyazev, 2012; Assenza & Di Lazzaro, 2015).

The delta wave slope, which reflects the amplitude and steepness of the slow waves observed in NREM sleep, can be considered a measure of synaptic plasticity given its close association with the firing synchronicity and efficacy of neuronal interaction (Knyazev, 2012; Assenza & Di Lazzaro, 2015). Specially, it has been shown that a steeper slope of delta wave activity indicates stronger synaptic connections, which are typically observed after periods of learning when synaptic potentiation occurs (Huber et al., 2004; Marshall et al., 2006; Ngo et al., 2013). Secondly, as synaptic downscaling occurs during sleep, there is a corresponding reduction in the delta wave slope, mirroring the decrease in synaptic strength and density (Riedner et al., 2007). Furthermore, studies involving learning tasks have shown that increased delta wave slope during subsequent sleep correlates with better performance and retention of the learned material (Huber et al., 2004). This suggests that the slope of delta waves could be indicative of the synaptic changes that occur due to learning and memory consolidation. Finally, various experimental interventions, such as pharmacological manipulations or sensory stimulation during sleep, have been used to alter delta wave characteristics. Consistent findings show that enhancing delta waves (e.g., through auditory stimulation) can lead to improved memory performance, supporting the idea that the delta wave slope is a marker of synaptic strength (Marshall et al., 2006; Ngo et al., 2013). Based on these evidences, the measurement of changes in delta wave slope provides researchers with an indirect yet effective means of inferring the extent of synaptic homeostasis that takes place during sleep.

We hope this explanation clarifies why delta slope can serve as an indirect indicator of changes in synaptic strength and density during sleep. We have included a brief explanation in the revised manuscript to ensure clarity for all readers (refer to lines 100 to 108, from page 2 to page 3).

In the revised manuscript:

“The process of synaptic homeostasis is reflected in delta wave activity^{45, 46}, which has been suggested to be generated by near-synchronous oscillations of large groups of neurons at the cellular level^{47, 48}. The slope of delta waves, which reflects their amplitude and steepness, is closely tied to the firing synchronicity and efficiency of neuronal interactions^{45, 46}. A steeper slope corresponds to stronger synaptic connections, typically observed after learning and synaptic potentiation^{2, 49, 50}. As synaptic downscaling occurs during sleep, there is a corresponding reduction in the slow wave slope, mirroring the decrease in synaptic strength and density⁵¹.”

References:

Borbély, A. A. A two process model of sleep regulation. Human Neurobiology 1, 195-204 (1982).

Borbely, A. A. From slow waves to sleep homeostasis: New perspectives. Archives italiennes de biologie 139, 53-61 (2001).

Tononi, G. & Cirelli, C. Steep function and synaptic homeostasis. Sleep Med. Rev. 10, 49-62 (2006).

Knyazev, G. G. EEG delta oscillations as a correlate of basic homeostatic and motivational processes. Neurosci. Biobehav. Rev. 36, 677-695 (2012).

Assenza, G. & Di Lazzaro, V. A useful electroencephalography (EEG) marker of brain plasticity: delta waves. Neural Regen Res 10, 1216-1217 (2015).

Kurth, S. et al. Characteristics of sleep slow waves in children and adolescents. Sleep 33, 475-480 (2010).

Riedner, B. et al. Sleep Homeostasis and Cortical Synchronization: III. A High-Density EEG Study of Sleep Slow Waves in Humans. Sleep 30, 1643-57 (2007).

Huber, R. et al. Local sleep and learning. Nature 430, 78-81 (2004).

Marshall, L. et al. Boosting slow oscillations during sleep potentiates memory. Nature 444, 610-3 (2006).

Ngo, H. V. et al. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. Neuron 78, 545-53 (2013).

Q10: The authors contrast SO (slow oscillations 0.2–1.7 Hz) with delta waves (1–4 Hz); these phenomena are partially overlapping in frequency, so it is not possible to separate their functions. Such a dissociation would necessitate non-overlapping frequencies and computing coupling of ripples, etc to both of the candidate low frequency modulations and seeing which one is more pronounced. This would be a worthwhile enterprise and would enhance the usefulness of the present study.

Response: Thank you for bringing this to our attention. In our original manuscript, we did not particularly focus on slow oscillations (SO), and only compared our delta-related findings with those related to SO from previous literature in the discussion section. However, we appreciate

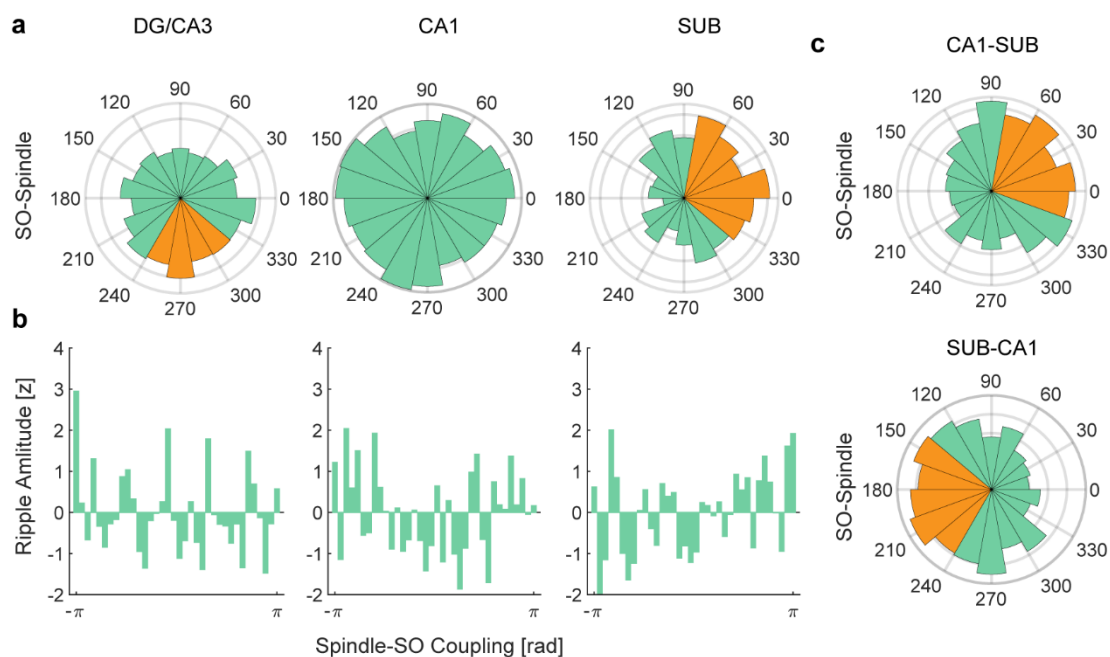
your comments about the importance of SO. Therefore, to investigate further, we conducted an analysis of SO-spindle and SO-spindle-ripple in the event-based coupling section, which is similar to the analysis done for the delta wave. To ensure a clear separation from delta wave, we strictly limited the SO range to less than 1Hz (0.2–1Hz) in our analysis. Overall, the results indicated that the coupling of SO-spindle within hippocampus was weaker than that of delta-spindle. Specially, unlike the observed triple coupling between delta waves, spindles and ripples in the CA1 field, no significant SO-spindle coupling was observed in the CA1 subfield (Supplementary Fig. 4a, CA1). Furthermore, we found no correlation between the phase of SO-spindle and ripple power in any subfield (Supplementary Fig. 4b). Moreover, even in cases where coupling was present, the coupling angles associated with SO differed from those of delta waves (Supplementary Fig. 4a, DG/CA3 and SUB; Supplementary Fig. 4c).

These findings align with previous research, as discussed in the manuscript's discussion section, indicating that while SO-dominated coupling occurs in the hippocampus during NREM sleep, it is weaker compared to delta-related coupling. Further investigation is warranted to elucidate the specific functions and competitive or complementary relationships between SO and delta slow waves.

We have included relevant descriptions regarding these analysis in the revised manuscript. Please refer to lines 206 to 213 on page 9.

In the revised manuscript:

“Previous studies have shown that there is an event-based coupling of SO-spindle in the hippocampus during NREM sleep²¹. We thus analyzed the SO-spindle coupling in each hippocampal subfield here. To avoid overlap with the delta frequency, the frequency range of SO here is limited to 0.2–1Hz. Unlike the observed delta-spindle-ripple coupling in the CA1 field, we found that there was no substantial coupling between SO and spindles within the CA1 subfield (Supplementary Fig. 3a). In addition, no other subfield was observed to show any modulation of SO–spindle phase on ripple power (Supplementary Fig. 3b).”



Supplementary Fig. 3 SO-spindle-ripple coupling in hippocampal subfields. **a** Normalized histogram (18 bins, 20° each) of preferred SO-spindle modulation phases across all detected spindle events (yellow bars represent significant coupling phase ranges; *V*-test, Bonferroni correction, $p < 0.05$). **b** Ripple activity in any subfield was not regulated by SO-spindle coupling phase (correlation between one circular and one linear random variable). **c** Similar to delta-spindle, the SO-spindle inter-regional coupling only manifests between CA1 and SUB (yellow bars represent significant coupling phase ranges; *V*-test, Bonferroni correction, $p < 0.05$).

Reviewer #2 (Remarks to the Author):

Q11: Li et al. present a study on oscillatory activity in the hippocampus during NREM sleep with a focus on the synaptic plasticity. A strength of the study is the relatively large sample and that the authors use intracranial recordings. The authors record from the CA3, CA1 and SUB regions. The authors report for a strong coupling between delta band activity in ripples in the places (especially DG/CA3). Especially in the CA1 and SUB fields there was strong coordination between activities. The study is of interest given the role memory consolidation processes in NREM sleep. The introduction is very balanced and in-depth and the study question is clear and relevant. The placement of the electrodes seems accurate and was based on the MR-navigation during surgery. From a methodological point of view, the study also seems sound (i.e. PAC was utilized using the appropriate implementation). The authors perform a multi-level analysis of spindle-ripple couplings and this line-up of these analyses is well-motivated. It is easy to follow the results section and the figures are clear. The same goes for the discussion section. My only minor point here is that the authors present the discussion in sections making it a bit difficult to fully comprehend the overarching picture of the study and the advance presented. I ask the authors to provide another structure in the discussion.

Response: We want to express our gratitude for your valuable feedback on our study. In response to your feedback, we have revisited the discussion section and restructured it to make it more understandable and emphasize the key findings of our research. We have kept the sectioned structure to maintain a clear and organized flow to the discussion. However, we have trimmed sections that may have obscured the main takeaways of our research and arranged the remaining sections in a concise and coherent manner.

In particular, we have extended our discussion to three major points. Firstly, we have elaborated on our findings which suggest that comparing to slow oscillations, delta rhythms dominate in the human hippocampus during NREM sleep, which laying the ground for the intricate coordination between delta wave with spindle and ripple oscillations. Secondly, we have trimmed two sections discussed in our previous manuscript ('Delta-ripple coupling in the DG/CA3' and 'Delta-spindle-ripple triple coordination in CA1') and integrated them under a new section titled 'Global versus local processing in the DG/CA3 and CA1' to highlight the complementary roles of distinct rhythmic synchronization patterns in the DG/CA3 and CA1 in global and local information processing. Finally, in the last section ('Hippocampal plasticity is differentially modulated by spindle and ripple events'), we discussed the differential role of spindle and ripple events in modulating synaptic plasticity during NREM sleep. Based on our findings, we argued that spindles may serve as a temporal window critical for synaptic upscaling, while ripples play a more precise regulatory role in initiating the upscaling process.

375 We hope that these changes have addressed your concern and improved the overall quality of
376 the manuscript. Thank you once again for your thoughtful feedback.

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

I have checked the reply of the authors to my comments, and i feel that they have fully addressed the concerns i raised in my original review.

1 Reviewer #1:

2 Q1: The manuscript does not have page or line numbers which complicates the review.

3 Response: We apologize for this oversight. Page and line numbers have now been included in
4 the revised manuscript to facilitate easier navigation and referencing during the review process.

5 Q2: The analysis is based on aggregate data from a number of subjects; it would be important
6 to add information related to single subjects in a revision. Major aspects of the analysis should
7 document variability between subjects.

8 Response: Thank you for bringing this issue to our attention. In response to the specific
9 questions posed (Q6 and Q7), we have included additional information about individual
10 subjects for our main results.

11 Q3: The manuscript is not hypothesis driven; the authors performed an exhaustive set of
12 analyses which is linked to some previous findings in the literature during the discussion. A
13 more directed, hypothesis-driven, approach would be much preferable, with close focus on the
14 main important and specific findings.

15 Response: Thank you for your insightful feedback on our manuscript. We appreciate your
16 observation that our study appears to lack a hypothesis-driven approach, and we agree that it
17 would be beneficial to focus more closely on specific research findings. While we performed
18 an exhaustive set of analyses to consider various potential factors influencing our study, we
19 acknowledge that a hypothesis-driven approach would have been more precise. Thus, we have
20 made thorough revisions to our manuscript, particularly in the introduction, results, and
21 discussion sections. These revisions result in a greater emphasis on our primary findings,
22 focusing on the intricate coupling of delta, spindle and ripple oscillations among the
23 hippocampal subfields and the distinct modulation of synaptic changes by hippocampal
24 spindles and ripples during NREM sleep.

25 In the introduction section, we have revised the presentation of our scientific questions. In
26 contrast to the original manuscript, we now explicitly state two main hypotheses as follows:

27 *In lines 87 to 95 on page 2:*

28 *“Given that oscillatory coupling has been established as a critical mechanism for interregional*
29 *information transmission in the brain^{18, 32, 33}, we hypothesize that information coordination*
30 *within the hippocampal circuit can be achieved through tight coupling among the cardinal*
31 *NREM oscillations between different hippocampal subfields. Moreover, increasing evidence*
32 *has suggested that each hippocampal subfield plays a unique role in processing information*
33 *essential for memory formation and consolidation^{26, 34-37}. Thus, we hypothesize further that*
34 *different hippocampal subfields may exhibit distinct profiles of sleep oscillation coupling*
35 *during NREM sleep.”*

36 *In line 108 to 116 on page 3:*

37 *“While the delta wave activity has been considered an indirect but effective marker of synaptic*
38 *plasticity and homeostasis, the other sleep events including spindle and ripple have been*
39 *suggested to provide crucial time windows for synaptic reshaping. However, there is a lack of*

research investigating the specific impact of hippocampal spindle and ripple oscillations on synaptic potentiation during NREM sleep. We thus hypothesize that hippocampal spindles and ripples may have distinct roles in regulating synaptic potentiation, which could be reflected in their associations with changes in delta-wave slope during NREM sleep.”

By directly pinpointing the core issues, we aim to clarify the focus of our study and enhance the clarity of our conclusions.

In the results section, to focus the manuscript more on our primary findings, we have removed the part titled “Weak delta-band synchrony within hippocampus during NREM”, as it did not closely align with our main discoveries. Moreover, we have restructured the discussion section to improve its coherence, making it more accessible and emphasizing our study’s pivotal discoveries.

Q4: NREM epochs were selected as showing prolonged elevations in the low delta band, reduced high gamma and elevated spindle activity. Were all of these criteria applied in the selection procedure? Which hippocampal subfield was analyzed for this selection; did all subfields have to show the 3 criteria to pass threshold? Please report mean and std err of NREM episode length across subjects.

Response: We apologize for the lack of detail in explaining the selection process for NREM epochs. To select NREM epochs, we followed the methodology used in previous studies (Jiang et al., 2017, 2020), which utilized SEEG data for NREM detection. In line with Jiang et al., we followed three criteria during the selection process: prolonged elevations in the low delta band, reduced high gamma activity, and elevated spindle activity. All the three criteria should be met when selecting NREM epochs.

Furthermore, since these criteria are primarily observed in neocortical regions, we focused on electrodes located in the neocortex rather than those in the hippocampus when selecting NREM epochs. As the number and placement of SEEG electrodes differ among participants, the specific neocortical electrodes used for NREM detection varied between individuals. However, to ensure the validity and accuracy of our NREM detection, for each participant, we only selected epochs in which the vast majority of the neocortical electrodes met all three criteria.

Additionally, we further ensured the accuracy of the selected NREM epochs by manually reviewing the SEEG waveforms and retaining only the epochs for which we had high confidence in our NREM sleep determination. As a result, the NREM epochs included in our analysis are relatively shorter in duration but were chosen with a focus on maintaining a high level of confidence in their accuracy.

In the revised manuscript, we have included these details in the relevant sections (please see lines 130 to 135 on page 5, and lines 498 to 450 on page 29). We have also provided the mean and standard error of NREM episode length across subjects in Supplementary Table 1 of the manuscript.

In the revised manuscript:

“Due to the lack of clinical sleep hypnograms, patient monitoring videos, scalp EEG, and electrooculograms, we selected NREM sleep (including N2 and N3 stages) based on neocortical

iEEG signals by identifying prolonged elevations in low delta band (<2 Hz) activity amplitude and corresponding declines in high-gamma (HG; 70–190 Hz) and initially high but gradually decreasing spindle band amplitudes (Fig. 1a).”

“When selecting NREM epochs for sleep staging, we focused solely on the electrodes located in the neocortex. NREM sleep was identified only if the majority of these neocortical electrodes met the aforementioned criteria.”

SUBJECT	DURATION OF RECORDING [H]	NUMBER OF NREM EPOCHS	DURATION OF NREM [MIN]	AVERAGE NREM DURATION PER EPOCH [MIN]
1	28	6	102	17
2	6.1	3	75	25
3	31.8	4	60	15
4	3.7	4	63	15
5	6	5	116	23
6	24	3	84	28
7	39.2	6	153	25
8	16	2	60	30
9	30	4	60	15
10	32	2	33	16
11	48	4	167	41
12	7.7	5	96	19
13	5.8	6	201	33
14	48	6	171	28
15	5.8	5	39	7
16	6.2	3	63	21
17	29.5	2	30	15
18	2	2	30	15
19	8.3	6	158	26
20	21.4	4	111	27
21	23.9	4	84	21
22	6.1	2	54	27
23	32	2	39	19
24	39	2	45	22
25	39.2	3	51	17
GROUP AVERAGE (MEAN ± SD)	21.588 ± 14.7	3.8 ± 1.5	85.8 ± 48.7	21.88 ± 7.2

Supplementary Table 1 Sleep metrics of each subject. Participants underwent continuous recording for several days, from which we selected several nights of sleep. Due to the

conservative approach, the extracted NREM (including N2 and N3, but excluding N1) sleep duration was relatively shorter.

References:

Jiang, X. et al. Replay of large-scale spatio-temporal patterns from waking during subsequent NREM sleep in human cortex. *Sci. Rep.* 7, 17380 (2017).

Jiang, X. et al. Improved identification and differentiation from epileptiform activity of human hippocampal sharp wave ripples during NREM sleep. *Hippocampus* 30, 610-622 (2020).

Q5: It is stated as a result that high delta and spindle frequency power were observed in NREM sleep, but these are the criteria used for selection! Reporting effects for which sites were selected is circular. Please explain clearly the logic of data analysis while avoiding such confounds.

Response: Thank you for your comment. We understand that it can lead to confusion as our previous manuscript didn't mention clearly that only neocortical electrodes are used for NREM sleep identification. We have included the details of NREM sleep selection in the revised manuscript (also see our response to Q4), which should make it clear that only neocortical electrodes were used for this process.

To further clarify, while we ensured high delta and spindle activity in the selection of NREM sleep, the electrodes used were from the neocortex rather than the hippocampus. Thus, the spectral properties of the hippocampus in NREM sleep still requires further investigation.

Furthermore, in this section, we also compared the spectral differences among hippocampal subfields to investigate if different subfields displayed different spectral profiles. We analyzed the spectral power of different frequency bands in different hippocampal subfields and found significant differences between certain subfields. Specifically, we found that the DG/CA3 showed an overall trend of higher power than the CA1, and SUB, and significant in 10–11 Hz and 27–30 Hz; But after normalization, the spindle band of SUB was significantly higher than that of DG/CA3. This analysis provided insights into the heterogeneity of hippocampal activity during NREM sleep. To more accurately reflect our focus, we have modified the title of this section in the revised manuscript to “Spectral profiles of different hippocampal subfields during NREM sleep”. We hope these clarifications help to better convey the findings of the section.

Our modification in the results section is line 127 on page 5.

In the revised manuscript:

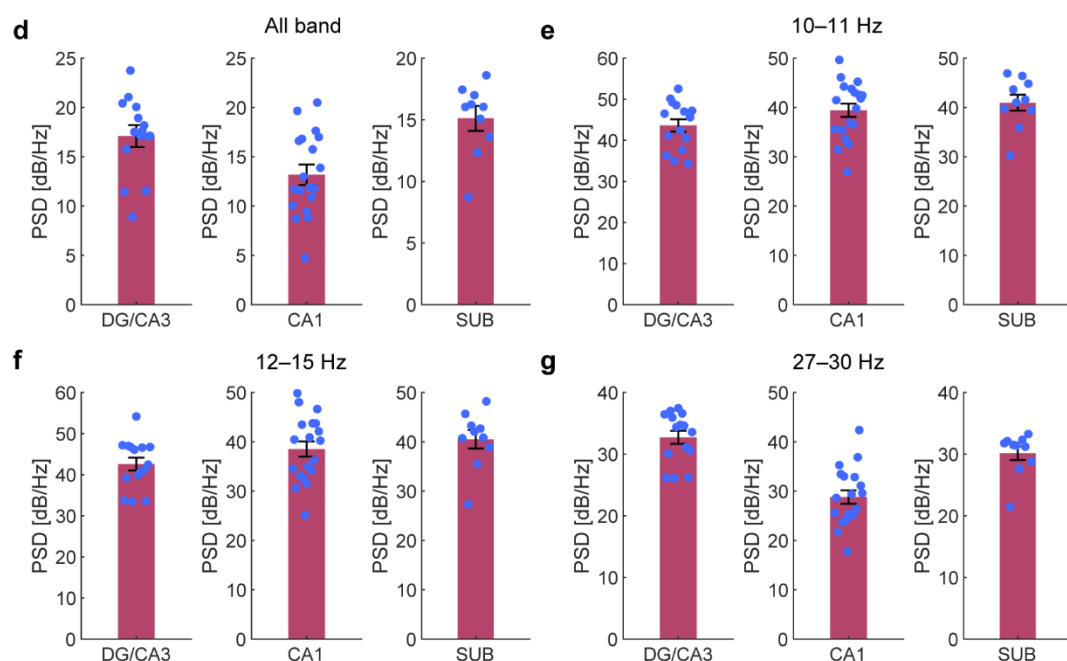
“Spectral profiles of different hippocampal subfields during NREM sleep”

Q6: The inset of Fig1. b is unreadable. What exactly is a “cluster correction”? The authors seem to have employed a multitude of *t*-tests, where great care must be taken to correct for multiple comparisons. The data as presented appear a little anecdotal. Please show main effects as scatter plots including all subjects, so the between subject variability can be examined.

Response: Thank you for your comments. We apologize for the imprecise wording used to describe the “cluster correction” method. Our intention was to refer to the commonly employed

cluster-based permutation correction method in the analysis of EEG/MEG/SEEG data. We have revised the manuscript accordingly, replacing the term “cluster correction” with “cluster-based permutation test”. Because MEG/EEG/iEEG data usually involves a substantial number of channel/time/frequency-pair comparisons, it is impossible to use traditional multiple comparison methods for correction. To address this issue, the cluster-based permutation test has been suggested as a solution. More information about the specific principles of this method can be found on the FieldTrip official website at https://www.fieldtriptoolbox.org/tutorial/cluster_permutation_timelock/.

To improve the presentation of our data, we have added scatter plots displaying the spectral results from all individual subjects to Supplementary Fig. 2d–g. These scatter plots provide a more comprehensive view of the data at an individual level. As can be seen from these figures, there are no substantial individual variations in the power of each band.



Supplementary Fig. 2d Scatter plots of the spectral mean in the three hippocampal subfields for the frequency range of 0 – 200 Hz. The bar and error bar plots represent the mean and SEM across all subjects, and blue dots represent individual subjects. **e – g** Similar to **d**, scatter plots of spectral means in three specific frequency bands (10 – 11 Hz, 12 – 15 Hz, and 27 – 30 Hz), which exhibit significant differences between hippocampal subfields.

Q7: Fig. 2b: there is no apparent enhancement in theta range for DG/CA3 vs. SUB, yet the statistical analysis reveals this. Please explain. Showing data for single subjects would be desirable also here.

Response: Thank you for bringing this to our attention. Upon re-examining the data, we have observed relatively high individual variability in phase-amplitude coupling values for the SUB region (see figure below), which may account for why there is “no apparent enhancement in the theta range, despite statistical analysis revealing differences”. Therefore, in the revised manuscript, we have removed the analysis comparing PAC values between hippocampal subfields to prevent inaccurate statistical results.

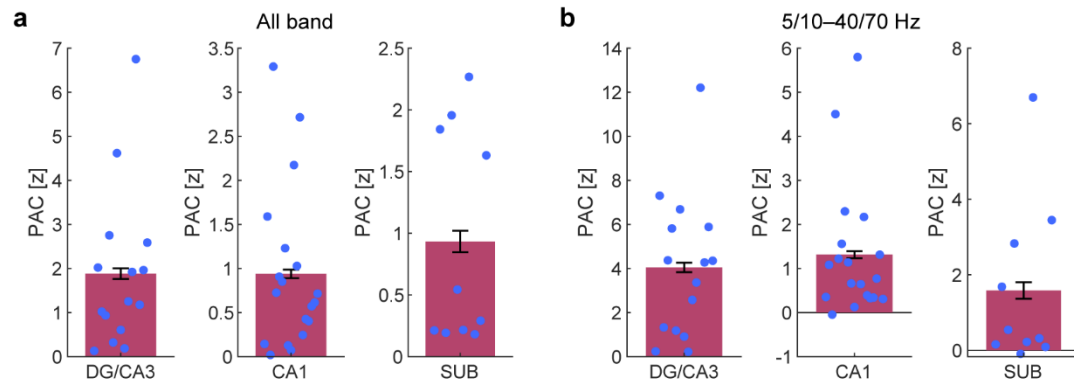


Figure 1. Scatter plots of the phase-amplitude coupling (PAC) averaged across (a) all frequency bands and (b) theta band (5/10–40/70 Hz) of three hippocampal subfields. The bars and error bars represent the mean and SEM across subjects, while the blue dots indicate individual subjects.

Q8: Fig.3: There is no systematic relationship between coupling phase in the three subfields. Please explain in more detail how these significant coupling phases are computed. The findings suggest different mechanisms are present in the three subfields for these couplings which seems unlikely.

Response: Thank you for your valuable comments. We estimated the significance of coupling phases among delta/SO, spindle and ripple oscillations according to previous research (Staresina et al., 2015). Specifically, as detailed in the methods section, we first filtered the raw signals for the frequencies of interest (SO: 0.2–1 Hz; delta: 1–4 Hz; spindle: 12–16 Hz; ripple: 80–100 Hz) within the spindle event epoch. We then used the Hilbert transform to extract the phase values of the lower-frequency oscillation (modulating) and the phase values of the power fluctuations of the higher-frequency oscillation (modulated). Next, we computed the coupling phase by evaluating the angle of synchronization index (SI; a complex number) between pairs of phase time series (modulating and modulated) for each event epoch. For the statistical analysis of phase angles, the V test, which assesses the non-uniformity of circular data with a specified mean direction, serves as a robust alternative to the Rayleigh test (Berens et al., 2009). For the statistical analysis of phase angles, we used the V test, which serves as a powerful alternative to the Rayleigh test, to test for non-uniformity of circular data with a specified mean direction (Berens et al., 2009). We pooled the resulting phase values across all participants and assessed whether certain phase values were significantly overrepresented compared to a uniform distribution, followed by Bonferroni multiple comparison correction.

We appreciate your comment regarding the possible presence of different mechanisms for the couplings in different hippocampal subfields. We would like to clarify that while the DG/CA3, CA1, and SUB all belong to the hippocampal formation, it does not necessarily imply that they cannot have different functions or processing mechanisms. In fact, emerging evidence has suggested that different hippocampal subfields may have distinct functions and processing modes. We would like to highlight some of these evidences. Firstly, the projections among the hippocampal subfields (DG, CA3, CA1, and SUB) form intricate and highly organized circuits. For example, the DG receives inputs from EC and projects to CA3, while CA1 receives inputs

from both EC and CA3 and projects to SUB as well as back to EC. These distinct projection patterns among the hippocampal subfields may underlie their different processing modes and roles in memory formation and consolidation. Secondly, prior research has shown that different hippocampal subfields play different roles in learning and memory processes (Gilbert et al., 2001; Lee & Kesner, 2002; Lee et al., 2004; Lasztóczy & Klausberger, 2017; Meier et al., 2020). For instance, the DG has been shown to have a pattern separation role while CA3/CA1 subfields are involved in pattern completion (Mizumori et al., 1989; Bakker et al., 2008). Moreover, previous studies have found a dissociation of the function in DG/CA3 and CA1 in terms of the NMDA receptor and backward shift in place fields in hippocampal-dependent memory task (Lee & Kesner, 2002; Lee et al., 2004), suggesting that DG/CA3 plays a key role in the rapid formation of representations of new information, whereas CA1 may be more important for comparing current experience with stored representations in the CA3 network. Furthermore, different neural oscillatory patterns, including frequency distribution, temporal organization and cross-frequency coupling have been found in DG/CA3 and CA1 areas (Lasztóczy & Klausberger, 2017; Meier et al., 2020), suggesting that network operations could be segregated in different hippocampal subfields. Based on all the available evidence, we believe that it is reasonable to postulate the existence of different mechanisms for different hippocampal subfields, which can be reflected by their distinct rhythmic synchronization patterns.

Our results align with these previously identified functional distinctions between hippocampal subfields. Specifically, our results indicate extensive coupling in both time and space within DG/CA3, while CA1 exhibits the strongest delta-spindle-ripple event-based coupling compared to DG/CA3 and SUB. The key distinction between event-based coupling and band-based phase-amplitude coupling lies in timescale: event-based coupling reflects short-term coupling strength associated with specific events, whereas band-based phase-amplitude coupling represents an average over longer timescales. Thus, these different coupling patterns in the three hippocampal subfields indicate their dissociable roles in information routing and processing, where the DG/CA3 may process information in a more global manner, while the CA1 may handle more precise information processing that relies on intricate modulations to integrate current cortical input with previously stored representations in the DG/CA3. Overall, our findings offer further support for the idea that different hippocampal subfields have distinct functions and processing modes. In the revised manuscript, we have expanded our discussion to offer a more comprehensive explanation regarding the potential dissociation in the roles of hippocampal subfields, as evidenced by their distinct rhythm coupling patterns. Please see lines 287 on page 15 to line 342 on page 16.

References:

- Staresina, B. P. et al. Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat. Neurosci.* 18, 1679-1686 (2015).
- Berens, P. et al. CircStat: a Matlab Toolbox for Circular Statistics. *Journal of Statistical Software.* 31, 1-21 (2009).
- Gilbert, P. E. et al. Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. *Hippocampus.* 6, 626-636 (2001).

Lee, I. & Kesner, R. P. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. *Nat Neurosci.* 5, 162-8 (2002).

Lee, I. et al. A double dissociation between hippocampal subfields: differential time course of CA3 and CA1 place cells for processing changed environments. *Neuron.* 42, 803-15 (2004).

Lasztóczy, B. & Klausberger, T. Distinct gamma oscillations in the distal dendritic fields of the dentate gyrus and the CA1 area of mouse hippocampus. *Brain Struct Funct.* 222, 3355-3365 (2017).

Meier K, et al. Dentate Gyrus Sharp Waves, a Local Field Potential Correlate of Learning in the Dentate Gyrus of Mice. *J Neurosci.* 40, 7105-7118 (2020).

Q9: The authors refer to modulations in synaptic plasticity modulations; yet these were not directly measured; please specify how this inference is made.

Response: Thank you for your valuable comment. While we did not directly measure synaptic plasticity in our study, our approach involved monitoring changes in delta wave slope across NREM sleep. A steeper delta wave slope is indicative of stronger and more numerous synaptic connections. Conversely, a reduction in this slope during sleep suggests synaptic downscaling, where synaptic strength and density are pruned to maintain homeostasis. This method is widely accepted in the field as an indirect but effective measure of synaptic plasticity and homeostasis (Tononi & Cirelli, 2006; Kurth et al., 2010; Knyazev, 2012; Assenza & Di Lazzaro, 2015).

The delta wave slope, which reflects the amplitude and steepness of the slow waves observed in NREM sleep, can be considered a measure of synaptic plasticity given its close association with the firing synchronicity and efficacy of neuronal interaction (Knyazev, 2012; Assenza & Di Lazzaro, 2015). Specially, it has been shown that a steeper slope of delta wave activity indicates stronger synaptic connections, which are typically observed after periods of learning when synaptic potentiation occurs (Huber et al., 2004; Marshall et al., 2006; Ngo et al., 2013). Secondly, as synaptic downscaling occurs during sleep, there is a corresponding reduction in the delta wave slope, mirroring the decrease in synaptic strength and density (Riedner et al., 2007). Furthermore, studies involving learning tasks have shown that increased delta wave slope during subsequent sleep correlates with better performance and retention of the learned material (Huber et al., 2004). This suggests that the slope of delta waves could be indicative of the synaptic changes that occur due to learning and memory consolidation. Finally, various experimental interventions, such as pharmacological manipulations or sensory stimulation during sleep, have been used to alter delta wave characteristics. Consistent findings show that enhancing delta waves (e.g., through auditory stimulation) can lead to improved memory performance, supporting the idea that the delta wave slope is a marker of synaptic strength (Marshall et al., 2006; Ngo et al., 2013). Based on these evidences, the measurement of changes in delta wave slope provides researchers with an indirect yet effective means of inferring the extent of synaptic homeostasis that takes place during sleep.

We hope this explanation clarifies why delta slope can serve as an indirect indicator of changes in synaptic strength and density during sleep. We have included a brief explanation in the revised manuscript to ensure clarity for all readers (refer to lines 100 to 108, from page 2 to page 3).

In the revised manuscript:

“The process of synaptic homeostasis is reflected in delta wave activity^{45, 46}, which has been suggested to be generated by near-synchronous oscillations of large groups of neurons at the cellular level^{47, 48}. The slope of delta waves, which reflects their amplitude and steepness, is closely tied to the firing synchronicity and efficiency of neuronal interactions^{45, 46}. A steeper slope corresponds to stronger synaptic connections, typically observed after learning and synaptic potentiation^{2, 49, 50}. As synaptic downscaling occurs during sleep, there is a corresponding reduction in the slow wave slope, mirroring the decrease in synaptic strength and density⁵¹.”

References:

Borbély, A. A. A two process model of sleep regulation. Human Neurobiology 1, 195-204 (1982).

Borbely, A. A. From slow waves to sleep homeostasis: New perspectives. Archives italiennes de biologie 139, 53-61 (2001).

Tononi, G. & Cirelli, C. Steep function and synaptic homeostasis. Sleep Med. Rev. 10, 49-62 (2006).

Knyazev, G. G. EEG delta oscillations as a correlate of basic homeostatic and motivational processes. Neurosci. Biobehav. Rev. 36, 677-695 (2012).

Assenza, G. & Di Lazzaro, V. A useful electroencephalography (EEG) marker of brain plasticity: delta waves. Neural Regen Res 10, 1216-1217 (2015).

Kurth, S. et al. Characteristics of sleep slow waves in children and adolescents. Sleep 33, 475-480 (2010).

Riedner, B. et al. Sleep Homeostasis and Cortical Synchronization: III. A High-Density EEG Study of Sleep Slow Waves in Humans. Sleep 30, 1643-57 (2007).

Huber, R. et al. Local sleep and learning. Nature 430, 78-81 (2004).

Marshall, L. et al. Boosting slow oscillations during sleep potentiates memory. Nature 444, 610-3 (2006).

Ngo, H. V. et al. Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. Neuron 78, 545-53 (2013).

Q10: The authors contrast SO (slow oscillations 0.2 – 1.7 Hz) with delta waves (1 – 4 Hz); these phenomena are partially overlapping in frequency, so it is not possible to separate their functions. Such a dissociation would necessitate non-overlapping frequencies and computing coupling of ripples, etc to both of the candidate low frequency modulations and seeing which one is more pronounced. This would be a worthwhile enterprise and would enhance the usefulness of the present study.

Response: Thank you for bringing this to our attention. In our original manuscript, we did not particularly focus on slow oscillations (SO), and only compared our delta-related findings with those related to SO from previous literature in the discussion section. However, we appreciate

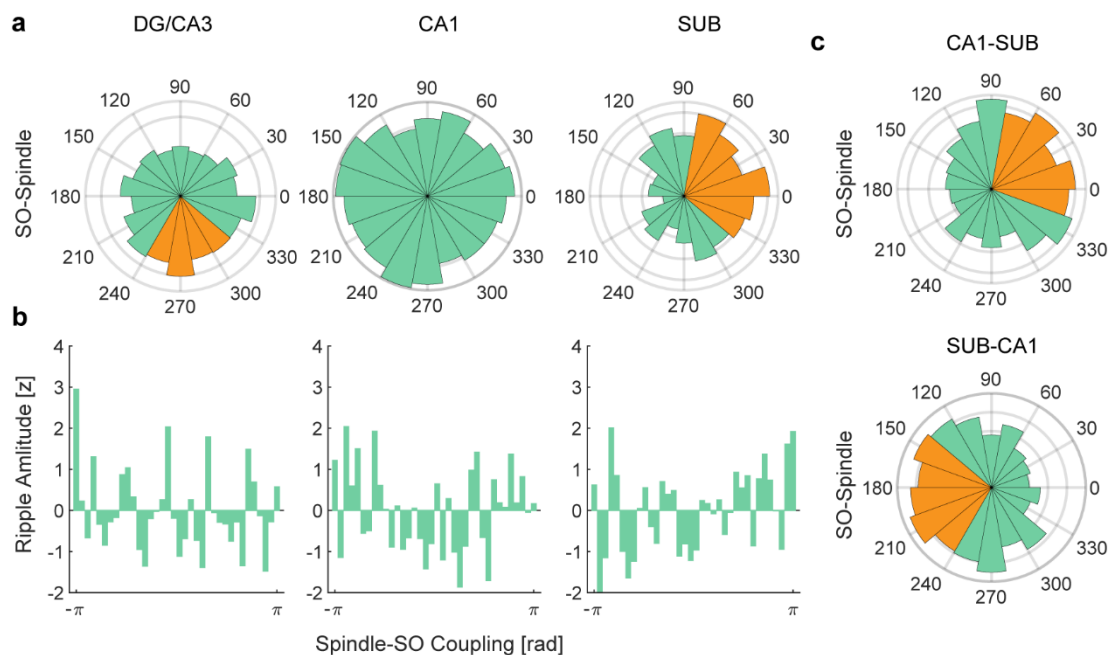
your comments about the importance of SO. Therefore, to investigate further, we conducted an analysis of SO-spindle and SO-spindle-ripple in the event-based coupling section, which is similar to the analysis done for the delta wave. To ensure a clear separation from delta wave, we strictly limited the SO range to less than 1Hz (0.2 – 1Hz) in our analysis. Overall, the results indicated that the coupling of SO-spindle within hippocampus was weaker than that of delta-spindle. Specially, unlike the observed triple coupling between delta waves, spindles and ripples in the CA1 field, no significant SO-spindle coupling was observed in the CA1 subfield (Supplementary Fig. 4a, CA1). Furthermore, we found no correlation between the phase of SO-spindle and ripple power in any subfield (Supplementary Fig. 4b). Moreover, even in cases where coupling was present, the coupling angles associated with SO differed from those of delta waves (Supplementary Fig. 4a, DG/CA3 and SUB; Supplementary Fig. 4c).

These findings align with previous research, as discussed in the manuscript’s discussion section, indicating that while SO-dominated coupling occurs in the hippocampus during NREM sleep, it is weaker compared to delta-related coupling. Further investigation is warranted to elucidate the specific functions and competitive or complementary relationships between SO and delta slow waves.

We have included relevant descriptions regarding these analysis in the revised manuscript. Please refer to lines 206 to 213 on page 9.

In the revised manuscript:

“Previous studies have shown that there is an event-based coupling of SO-spindle in the hippocampus during NREM sleep²¹. We thus analyzed the SO-spindle coupling in each hippocampal subfield here. To avoid overlap with the delta frequency, the frequency range of SO here is limited to 0.2 – 1Hz. Unlike the observed delta-spindle-ripple coupling in the CA1 field, we found that there was no substantial coupling between SO and spindles within the CA1 subfield (Supplementary Fig. 3a). In addition, no other subfield was observed to show any modulation of SO – spindle phase on ripple power (Supplementary Fig. 3b).”



Supplementary Fig. 3 SO-spindle-ripple coupling in hippocampal subfields. **a** Normalized histogram (18 bins, 20° each) of preferred SO-spindle modulation phases across all detected spindle events (yellow bars represent significant coupling phase ranges; *V*-test, Bonferroni correction, $p < 0.05$). **b** Ripple activity in any subfield was not regulated by SO-spindle coupling phase (correlation between one circular and one linear random variable). **c** Similar to delta-spindle, the SO-spindle inter-regional coupling only manifests between CA1 and SUB (yellow bars represent significant coupling phase ranges; *V*-test, Bonferroni correction, $p < 0.05$).

Reviewer #2 (Remarks to the Author):

Q11: Li et al. present a study on oscillatory activity in the hippocampus during NREM sleep with a focus on the synaptic plasticity. A strength of the study is the relatively large sample and that the authors use intracranial recordings. The authors record from the CA3, CA1 and SUB regions. The authors report for a strong coupling between delta band activity in ripples in the places (especially DG/CA3). Especially in the CA1 and SUB fields there was strong coordination between activities. The study is of interest given the role memory consolidation processes in NREM sleep. The introduction is very balanced and in-depth and the study question is clear and relevant. The placement of the electrodes seems accurate and was based on the MR-navigation during surgery. From a methodological point of view, the study also seems sound (i.e. PAC was utilized using the appropriate implementation). The authors perform a multi-level analysis of spindle-ripple couplings and this line-up of these analyses is well-motivated. It is easy to follow the results section and the figures are clear. The same goes for the discussion section. My only minor point here is that the authors present the discussion in sections making it a bit difficult to fully comprehend the overarching picture of the study and the advance presented. I ask the authors to provide another structure in the discussion.

Response: We want to express our gratitude for your valuable feedback on our study. In response to your feedback, we have revisited the discussion section and restructured it to make it more understandable and emphasize the key findings of our research. We have kept the sectioned structure to maintain a clear and organized flow to the discussion. However, we have trimmed sections that may have obscured the main takeaways of our research and arranged the remaining sections in a concise and coherent manner.

In particular, we have extended our discussion to three major points. Firstly, we have elaborated on our findings which suggest that comparing to slow oscillations, delta rhythms dominate in the human hippocampus during NREM sleep, which laying the ground for the intricate coordination between delta wave with spindle and ripple oscillations. Secondly, we have trimmed two sections discussed in our previous manuscript ('Delta-ripple coupling in the DG/CA3' and 'Delta-spindle-ripple triple coordination in CA1') and integrated them under a new section titled 'Global versus local processing in the DG/CA3 and CA1' to highlight the complementary roles of distinct rhythmic synchronization patterns in the DG/CA3 and CA1 in global and local information processing. Finally, in the last section ('Hippocampal plasticity is differentially modulated by spindle and ripple events'), we discussed the differential role of spindle and ripple events in modulating synaptic plasticity during NREM sleep. Based on our findings, we argued that spindles may serve as a temporal window critical for synaptic upscaling, while ripples play a more precise regulatory role in initiating the upscaling process.

375 We hope that these changes have addressed your concern and improved the overall quality of
376 the manuscript. Thank you once again for your thoughtful feedback.