nature portfolio

Peer Review File

Hippocampal ripples predict motor learning during brief rest breaks in humans

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Recent work documented that crucial aspects of motor learning and memory develop during periods of wakeful rest and sleep. Early behavioral gains when learning sequential motor skills develop during these rest intervals. The hippocampus appears to contribute to this form of learning. The authors here investigate a possible hippocampal mechanism that could contribute to this form of rapid consolidation of skill. They studied epilepsy patients (n=17) undergoing direct intracranial EEG monitoring of the hippocampus as they learned a motor sequence task. They report that, as shown before in healthy young individuals, patients developed skill gains to a larger extent over periods of rest rather than practice. Crucially, they demonstrated increased hippocampal ripple rates during these rest breaks that predicted offline gains in speed. These findings showed that hippocampal ripples could could contribute to rapid consolidation of skill during this procedural memory task, as has been previously shown in the context of declarative memory.

The paper is well written. The introduction is accurate, the results well-presented and the Discussion informative. A revised manuscript addressing the following issues could enhance the paper's impact.

Comments
1. The authors trained subjects using periods of 30s rest and practice. Previous work suggested that 30s intervals could favor the development of reactive inhibition, an issue minimized when implementing rest and practice intervals of 10s [1]. Have you tried using this approach, and thus minimizing the possible impact of motor fatigue? Are results with both protocols (10/10 and 30/30) the same? It would be useful to comment on possible differences in results between the two protocols.

2. The authors build a compelling argument for the contribution of hippocampal ripples during rest intervals of early learning to rapid consolidation of skill, consistent with work done in healthy individuals using MEG [2]. Importantly, their findings are strongly in line with work in a different lab [3]. Altogether, they provide strong mechanistic evidence for the contribution of wakeful neural replay during rest to early procedural learning.

3. In the discussion, the authors indicate that when the task is twice as fast, and thus more fatiguing (during screening non-learning conditions), online losses are about half of those in the learning, slower task. This, as the authors indicate is incompatible with a motor fatigue origin of online losses. This view is consistent with the finding that reduction of practice period duration in about half (and thus reduction of hypothetical fatigue) does not modify micro-offline learning [4], an issue the authors should add to the Discussion.

4. Perhaps more problematic is the authors proposal that micro-offline gains "…could represent recovery from "psychomotor fatigue". It would be important for the authors to define what do they mean by psychomotor fatigue and what evidence points for or against this interpretation (related to the previous point). How do the authors differentiate psychomotor fatigue from consolidation? What is the relationship between psychomotor fatigue and ripples?

In summary, the paper is an important contribution to the literature on hippocampal contributions to consolidation of early procedural motor skill learning in general and hippocampal ripples and neural replay in particular.

References

1. Pan, S.C. and T.C. Rickard, Sleep and motor learning: Is there room for consolidation? Psychol Bull, 2015. 141(4): p. 812-34.
2. Buch, E.R., et al., Consolidation of human skill linked to waking hippocampo-neocortical replay. Cell Rep, 2021. 35(10): p. 109193.
3. Chen, P.-C., et al., Hippocampal ripples during offline periods predict human motor sequence learning. bioRxiv, 2024: p. 2024.10.06.614680.
4. Bönstrup, M., et al., Mechanisms of offline motor learning at a microscale of seconds in large-scale crowdsourced data. NPJ Sci Learn, 2020. 5: p. 7.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The manuscript addresses a very important and timely topic: ultra-fast consolidation, which is currently a "hot topic" in memory research. The study presented in the manuscript convincingly demonstrates the role of the hippocampus using intracranial EEG. Both the experimental design and statistical analyses are well-planned and compelling. Using the "good cop, bad cop" terminology, my review falls into the "good cop" category.

My suggestions and comments are as follows:

Literature Review and Interpretation of Results

The introduction does not sufficiently cover the relevant literature, which impacts the interpretation of the results.

Procedural memory is not a monolithic concept. Different types of procedural memory may behave differently in terms of ultra-fast consolidation and online vs. offline learning. The authors examined only one procedural task, which is a limitation that should be acknowledged. For reference, see:
Quentin et al. (2021). Statistical learning occurs during practice while high-order rule learning during rest period. NPJ Science of Learning, 6(14).

The duration of the offline period is another critical factor, which the authors did not manipulate. This should be discussed when interpreting the results. Relevant studies include:
Szücs-Bencze et al. (2023). Manipulating the rapid consolidation periods in a learning task affects general skills more than statistical learning and changes the dynamics of learning. eNeuro, 10(2).
Fanuel et al. (2022). How does the length of short rest periods affect implicit probabilistic learning? Neuroimage: Reports, 2(1), 100078.

It is also important to note significant individual differences in whether participants are more inclined toward offline or online learning. Since the authors studied only 17 participants, individual differences could not be examined in depth, but this should still be considered in the discussion of the results. For reference, see:
Szücs-Bencze et al. (see above).

Furthermore, online and offline learning can differ significantly across various clinical populations. If ultra-fast consolidation is robust, it could even serve as a basis for therapeutic interventions. See:
Nagy et al. (2025). Intact ultrafast memory consolidation in adults with autism and neurotypicals with autism traits. Brain Research, 1847.

Methodological Issues
While the mini offline change measure appears reliable and supports the validity of the findings, the measure of online change within practice blocks is less robust. Specifically, this measure compares the performance at the very start of a block to the very end. A large difference is interpreted as significant online learning, while a small difference indicates minimal online learning.
However, it is possible (and common) for performance to peak mid-block, reflecting substantial online learning, but this may

not be captured due to noisy data or lower performance at the end of the block. Similarly, if the final few trials show strong performance, this could erroneously indicate significant online improvement. Conversely, poor performance in the final few trials may mask substantial online learning. Although this performance/competence issue does not detract from the overall value of the manuscript, it is crucial to inform readers about this limitation in the discussion. Specifically, the offline change measure is far more reliable than the online change measure, making direct comparisons between online and offline learning more tentative. Additionally, future research should aim to develop more robust methods for calculating online learning measures.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)
In this study, authors performed intracranial EEG recordings from the hippocampus of 17 epilepsy patients during a motor sequence learning task, and examined the relationship between the hippocampal ripples and the online and offline gains within one session of motor learning. Their results show that the ripple rates during offline periods following each block is correlated with the level of offline gains in motor performance on a subject-by-subject and a trial-by-trial basis. These results are very interesting, and could provide direct evidence for the involvement of hippocampal ripples in offline motor sequence learning in humans, which is currently missing in the literature. However, there are major limitations in the methods that make the interpretation of the observed results very difficult, as outlined in my comments below. Without the addition of a proper control condition the overall impact of this study is restricted.

Major comments:

1- My main concern is regarding the lack of a control condition to account for non-specific effects of motor learning. There are a variety of factors, including motor fatigue, use-dependent practice, task novelty, or cognitive fatigue that may confound the process of sequence learning. As authors mention in the Discussion, a necessary control condition is recording the hippocampal ripples, e.g., during performance of a simple sequence (1-2-3-4) over 12 blocks. They acquired some data only in 7 subjects, for only 2 blocks, and without neural recordings. They already report some motor fatigue related to this simple sequence. Without a proper control condition, and examining how the hippocampal ripples relate to similar measures of offline and online gains during this control condition, the impact of the reported results is not clear.

2- The lack of a control condition is exacerbated by the proposed definitions of online and offline gains in this study. Their definition of online gain is misleading, as it is vastly confounded by motor/cognitive fatigue during each trial, and the subject’s readiness at the beginning of each trial after each rest. In fact, authors show a negative effect of online learning! Also their definition of offline gain is equally confounded by these factors. Establishing any relationship with these within-session measures requires rigorous control conditions to account for non-specific effects of learning.

3- The main findings are reported in Figure 3 based on the main effect of ripples between offline and online periods, and their correlation with the gains. However, it seems that both 3A and 3B results are biased by 2 outliers (the 2 red points with high mean ripple rates). It should be clarified if the results still may hold after the removal of these outliers.

(Remarks on code availability)
Yes, the results of the paper are reproducible with the provided code resources.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Concerns from the first round of review have been satisfactorily addressed.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The authors have satisfactorily addressed my questions. I recommend the manuscript for publication.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)
The authors have now adequately addressed my concerns by analyzing the neural data during the screening task. They have also analyzed data using standard definitions of micro-online and offline gains. Overall the manuscript has substantially improved and I have no further comments/concerns.

(Remarks on code availability)
Supporting data and R code is provided.

We thank the reviewers for their thoughtful and constructive critiques and have extensively revised the manuscript and added several new analyses to address their concerns. Importantly, as suggested by Reviewer 4, we have added analyses of hippocampal ripples during the control (screening) task. These analyses support our hypothesis that the increase in ripples during offline periods and their correlation with micro-offline performance gains reflect that ripples mediate motor memory consolidation rather than non-specific factors related to motor performance.

Reviewer #1

1.0: *Recent work documented that crucial aspects of motor learning and memory develop during periods of wakeful rest and sleep. Early behavioral gains when learning sequential motor skills develop during these rest intervals. The hippocampus appears to contribute to this form of learning. The authors here investigate a possible hippocampal mechanism that could contribute to this form of rapid consolidation of skill. They studied epilepsy patients (n=17) undergoing direct intracranial EEG monitoring of the hippocampus as they learned a motor sequence task. They report that, as shown before in healthy young individuals, patients developed skill gains to a larger extent over periods of rest rather than practice. Crucially, they demonstrated increased hippocampal ripple rates during these rest breaks that predicted offline gains in speed. These findings showed that hippocampal ripples could contribute to rapid consolidation of skill during this procedural memory task, as has been previously shown in the context of declarative memory.*

The paper is well written. The introduction is accurate, the results well-presented and the Discussion informative. A revised manuscript addressing the following issues could enhance the paper's impact.

1.1: *The authors trained subjects using periods of 30s rest and practice. Previous work suggested that 30s intervals could favor the development of reactive inhibition, an issue minimized when implementing rest and practice intervals of 10s (Pan and Rickard, 2015). Have you tried using this approach, and thus minimizing the possible impact of motor fatigue? Are results with both protocols (10/10 and 30/30) the same? It would be useful to comment on possible differences in results between the two protocols.*

We have not tried a protocol with 10/10 s and, unfortunately, it was not feasible to perform additional experiments given our limited access to this acute inpatient sample and limits on their ability to tolerate participation. We now comment on our rationale for 30-s trials in this clinical population and the possible effects of longer trials on fatigue in the limitations paragraph of the discussion.

Discussion, lines 237-254:

“Several limitations of the present study are worth noting. Some prior studies of healthy young participants used shorter typing and rest periods (e.g., 10-s), which leave less time for fatigue to build.[6, 8, 31] We chose 30-s trials based on previous studies of clinical samples (including amnesia, schizophrenia, and children with epilepsy) and older healthy adults to account for slower typing speeds than young healthy subjects and to reduce set-switching demands.[9, 32, 33] Comparing participants in the present study with the young health adults (mean age 27) reported in Mylonas et al., 2024,[9] our epilepsy inpatients typed only about half as fast (2.18±0.88 vs. 4.49±1.76 keypresses/s; t_{37}=4.95, p=1.6x10-5) and took much longer to begin typing at the start of each trial (1193±810 vs. 711±490 ms; t_{37}=2.92, p=.006) suggesting larger set-switching costs. Regardless of whether previous studies used 5-s, 10-s, [6, 8, 31] or up to 30-s typing and rest periods,[7, 9] performance improved significantly during offline periods while online performance either decreased or was not significantly greater than zero. In a functional MRI study that used 30-s trials, hippocampal activation during rest predicted micro-offline gains.[7] Since hippocampal activity is not known to correlate with fatigue, this finding is consistent with the interpretation that offline gains, even with 30-s trials, reflect motor memory consolidation. It would be useful to manipulate the duration of typing and rest periods and to have more trials of a non-learning control task to disentangle the potential contributions of recovery from fatigue, memory stabilization, memory enhancement, and other factors to micro-offline gains and their relations to ripples.”

1.2 *The authors build a compelling argument for the contribution of hippocampal ripples during rest intervals of early learning to rapid consolidation of skill, consistent with work done in healthy individuals using MEG (Buch et al., 2021). Importantly, their findings are strongly in line with work in a different lab (Chen et al., 2024). Altogether, they provide strong mechanistic evidence for the contribution of wakeful neural replay during rest to early procedural learning.*

Thank you. We have extensively revised the manuscript to highlight these points and in response to the reviewers' suggestions and concerns.

1.3: *In the discussion, the authors indicate that when the task is twice as fast, and thus more fatiguing (during screening non-learning conditions), online losses are about half of those in the learning, slower task. This, as the authors indicate is incompatible with a motor fatigue origin of online losses. This view is consistent with the finding that reduction of practice period duration in about half (and thus reduction of hypothetical fatigue) does not modify micro-offline learning*,(Bonstrup et al., 2020) *an issue the authors should add to the Discussion.*

Thank you, we have added the following to the Discussion, lines 214-216:

"Previous findings that micro-offline gains are similar in magnitude even when typing periods are reduced by half, which should reduce fatigue, are also incompatible with 'recovery from fatigue' as the sole explanation of micro-offline gains.[14]"

1.4: *Perhaps more problematic is the authors proposal that micro-offline gains "…could represent recovery from "psychomotor fatigue". It would be important for the authors to define what do they mean by psychomotor fatigue and what evidence points for or against this interpretation (related to the previous point). How do the authors differentiate psychomotor fatigue from consolidation? What is the relationship between psychomotor fatigue and ripples?*

We define psychomotor fatigue as a decline in performance due to sustained mental and physical exertion. We agree that it is not a helpful term in this context and have removed it.

Although we cannot definitively differentiate the contributions of recovery from fatigue and memory consolidation to offline gains and their relationship to ripples, a limitation that we acknowledge in the discussion, we present new evidence from our results and from the literature that offline gains reflect memory consolidation. The limitations and new evidence are quoted below. We have also added Figure S3 to show the ripple data from the MST vs. screening task that are incompatible with a purely recovery from fatigue account of offline gains.

Results, lines 129-133:

"In a set of analyses we addressed the question of whether offline gains primarily reflect recovery from fatigue from the previous typing trial. If this were the case, one would expect more typing to be more fatiguing and to therefore result in greater online losses of speed (i.e., negative online gains). This was not what we found. The number of sequences typed did not correlate with online gains either across participants ($r=.13$, $p=.61$) or across trials (β est.=-0.01, CI: -0.05, 0.03, $p=.67$)."

Discussion, lines 205-223:

"Our data, however, indicate that the motor slowing seen during typing periods is unlikely to be due solely to fatigue. If it were, one would expect typing more sequences to be more fatiguing and to therefore result in greater online losses of speed (i.e., negative online gains). This was not the case: the number of sequences typed did not correlate with online losses either across participants or across trials. In addition, on a screening task that minimizes learning, participants typed almost twice as fast as on the MST yet showed less than half the online losses. The findings of no correlations of the number of sequences typed with motor slowing and that the same participants were capable of sustaining higher typing speeds on an over-learned sequence than on the MST are incompatible with a purely fatigue explanation of the online motor slowing during the MST. Findings that micro-offline gains are similar in magnitude even when typing periods are reduced by half, which should reduce fatigue, are also incompatible with 'recovery from fatigue' as the sole explanation of micro-offline gains.[14] Alternatively, we propose that micro-offline gains reflect motor memory consolidation during rest. This hypothesis is consistent with findings that the motor memory becomes more resistant to interference from learning a new sequence if the new sequence is presented after a brief delay (10 s) rather than immediately after typing.[14] This suggests that the stabilization of motor memory traces can happen over seconds. Although we cannot exclude contributions from non-specific factors related to motor performance (e.g., fatigue due to mental or physical exertion), collectively, these behavioral findings support the hypothesis that micro-offline gains reflect a rapid form of motor memory consolidation."

Discussion, lines 252-254:

"It would be useful to manipulate the duration of typing and rest periods and to have more trials of a non-learning control task to disentangle the potential contributions of recovery from fatigue, memory stabilization, memory enhancement, and other factors to micro-offline gains and their relations to ripples."

Methods, lines 395-398:

"We used a mixed effects model of the form OnlineGains ~ SequencesTyped + (1 + SequencesTyped | Subject) to evaluate whether the number of sequences typed was associated with online gains. If the observed online motor slowing were due to fatigue we would expect more sequences typed to correlate with lower or more negative online gains."

In summary, the paper is an important contribution to the literature on hippocampal contributions to consolidation of early procedural motor skill learning in general and hippocampal ripples and neural replay in particular.

Reviewer #3

3.0: The manuscript addresses a very important and timely topic: ultra-fast consolidation, which is currently a "hot topic" in memory research. The study presented in the manuscript convincingly demonstrates the role of the hippocampus using intracranial EEG. Both the experimental design and statistical analyses are well-planned and compelling. Using the "good cop, bad cop" terminology, my review falls into the "good cop" category.

3.1: *The introduction does not sufficiently cover the relevant literature, which impacts the interpretation of the results.*

Thank you for calling our attention to literature that is relevant to our interpretation of the findings. We have modified the manuscript to incorporate these suggestions, as described below.

3.2: *Procedural memory is not a monolithic concept. Different types of procedural memory may behave differently in terms of ultra-fast consolidation and online vs. offline learning. The authors examined only one procedural task, which is a limitation that should be acknowledged. For reference, see: Quentin et al. (2021). Statistical learning occurs during practice while high-order rule learning during rest period. NPJ Science of Learning, 6(14).*

Please see our response to **3.5** below.

3.3: *The duration of the offline period is another critical factor, which the authors did not manipulate. This should be discussed when interpreting the results. Relevant studies include: Szücs-Bencze et al. (2023). Manipulating the rapid consolidation periods in a learning task affects general skills more than statistical learning and changes the dynamics of learning. eNeuro, 10(2). Fanuel et al. (2022). How does the length of short rest periods affect implicit probabilistic learning? Neuroimage: Reports, 2(1), 100078. 1.2;3.2*

Please see our response to **3.5** below.

3.4: *It is also important to note significant individual differences in whether participants are more inclined toward offline or online learning. Since the authors studied only 17 participants, individual differences could not be examined in depth, but this should still be considered in the discussion of the results. For reference, see: Szücs-Bencze et al. (see above).*

Please see our response to **3.5** below.

3.5: O*nline and offline learning can differ significantly across various clinical populations. If ultra-fast consolidation is robust, it could even serve as a basis for therapeutic interventions. See: Nagy et al. (2025). Intact ultrafast memory consolidation in adults with autism and neurotypicals with autism traits. Brain Research, 1847.*

We now acknowledge these limitations along with the possibilities of individual and clinical population differences in rapid consolidation and cite the suggested literature in the Discussion, lines 255-260:

"Future studies might also establish whether the findings of rapid consolidation of motor sequence learning and its relation to hippocampal ripples generalize to other kinds of procedural learning (c.f., [34, 35] for differences in the role of rest breaks for statistical, rule, and implicit learning). There are also likely to be individual differences in the reliance on online vs. offline learning that the present study was underpowered to explore, [36] as well as differences among clinical populations that may reflect hippocampal function. [9, 34, 38, 39]"

3.6: *While the mini offline change measure appears reliable and supports the validity of the findings, the measure of online change within practice blocks is less robust. Specifically, this measure compares the performance at the very start of a block to the very end. A large difference is interpreted as significant online learning, while a small difference indicates minimal online learning.*

However, it is possible (and common) for performance to peak mid-block, reflecting substantial online learning, but this may not be captured due to noisy data or lower performance at the end of the block. Similarly, if the final few trials show strong performance, this could erroneously indicate significant online improvement. Conversely, poor performance in the final few trials may mask substantial online learning. Although this performance/competence issue does not detract from the overall value of the manuscript, it is crucial to inform readers about this limitation in the discussion. Specifically, the offline change measure is far more reliable than the online change measure, making direct comparisons between online and offline learning more tentative. Additionally, future research should aim to develop more robust methods for calculating online learning measures.

We adopted the measures of micro-online and offline gains that have been used in prior studies of the MST.[6-9, 14] If we substitute the average speed of the first three and last three sequences for the speed of the first and last sequences in these calculations, which should reduce the influence of transient fluctuations in performance, the results are substantively the same (See Table R1 and Figs. R1, R2 below).

As described our response to Reviewer 1, Critiques **1.1**, **1.3**, and **1.4**, the Results and Discussion now extensively address the potential contributions of fatigue and other non-specific factors related to motor performance and the need for future work to disentangle them.

	First & last sequence	Average of first 3 & last 3
Total gains vs zero	t_{16}= 3.41, p=.004	t_{16}= 4.15, p=.0008
Offline gains vs zero	t_{16}= 3.69, p=.002	t_{16}= 3.75, p=.002
Online gains vs zero	t_{16}= -2.59, p=.02	t_{16}= -2.50, p=.025
Offline > online gains	t_{16}=3.13, p=.006	t_{16}=3.51, p=.003
*Gains ~ Ripple Rate * Period + (1 + Ripple Rate | Subject)*		

Ripple Rate	Est.= 2.38, p= .0008	Est.= 1.21, p= .006
Interaction: RippleRate - Period	Est.= -3.11, p= .0001	Est.= -1.29, p= .019
OfflineGains ~ Offline Ripple Rate + (1 + Ripple Rate | Subject)		
Offline Ripple Rate	Est.= 2.26, p= .002	Est.= 1.05, p= .0096
OnlineGains ~ Online Ripple Rate + (1 + Ripple Rate | Subject)		
Online Ripple Rate	Est.= -0.73, p=.40	Est.= -0.11, p= .84
Corr: Mean offline ripple rate vs offline gains	r=.64, p=.005	r=.67, p=.003
Corr: Mean online ripple rate vs mean online gains	r=.006, p=.98	r=.10, p=.72

Table R1: Comparison of relevant statistics when using the speed during the first and last sequence in a trial to calculate gains vs using the average speed of the first 3 and last 3 sequences in a trial to calculate gains.

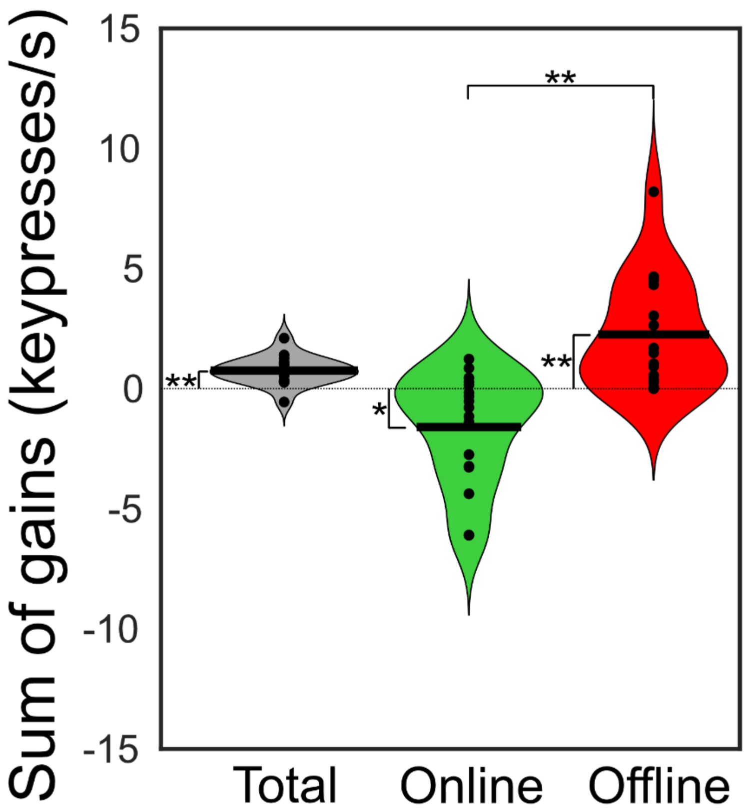

Figure R1: Violin plots showing distribution of the sum of total gains, micro-online gains and micro-offline gains calculated the average speed of the first 3 and last 3 sequences in a trial across 11 trials across participants. The black dots represent participants' means and horizontal lines represent group means. Significant differences are indicated with asterisks (* = p<.05, ** = p<.005). Please compare to Figure 1A, right.

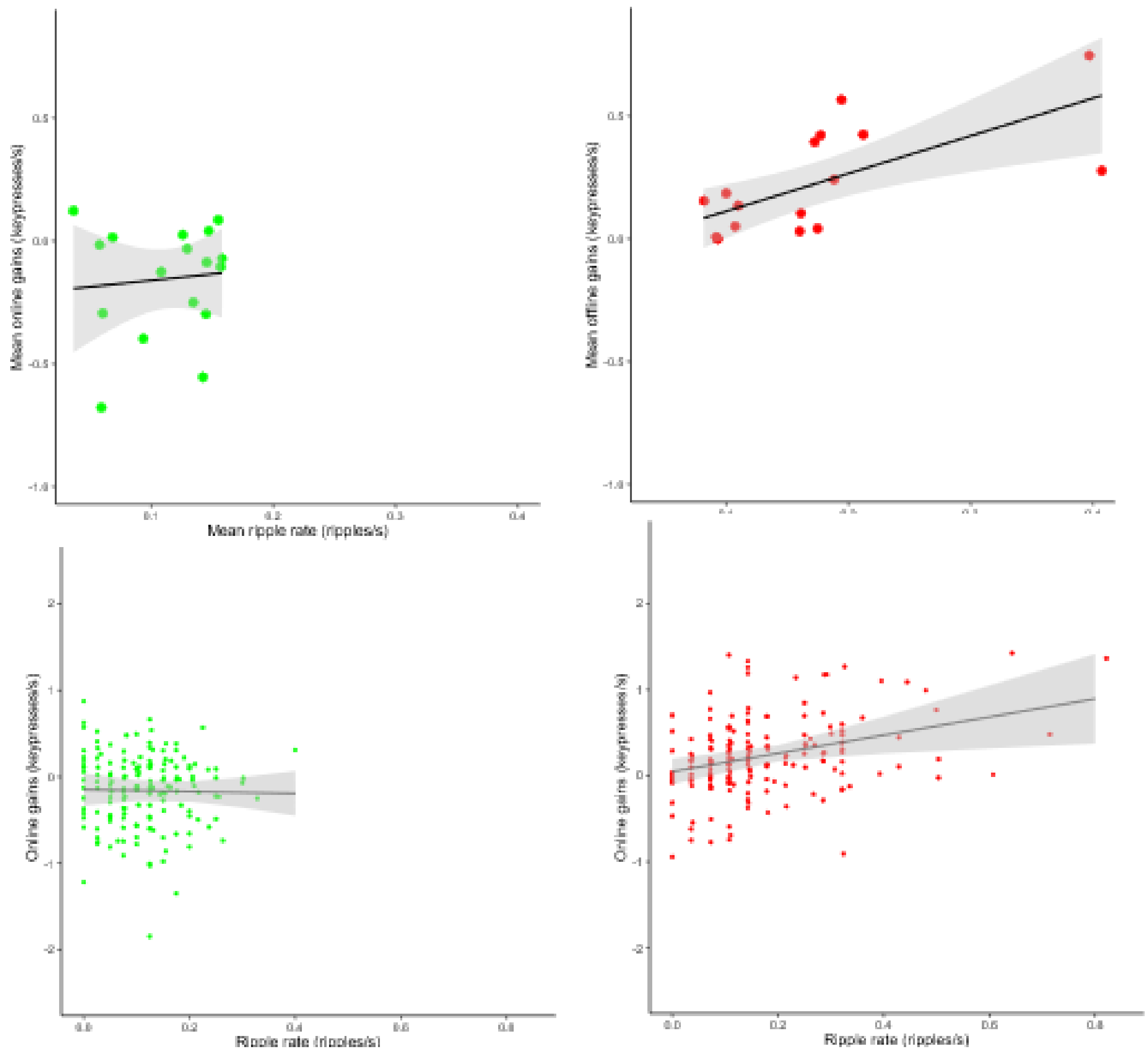

Figure R2: *Top row*: Scatter plots of mean online (left) and offline (right) ripple rate and gains across trials using the average speed of the first 3 and last 3 sequences in a trial to calculate gains. Each participant is a circle. The shading represents the standard error of the regression line. *Bottom row:* Scatter plots of ripple rates and gains per trial for each participant.

Reviewer #4

4.0: *In this study, authors performed intracranial EEG recordings from the hippocampus of 17 epilepsy patients during a motor sequence learning task, and examined the relationship between the hippocampal ripples and the online and offline gains within one session of motor learning. Their results show that the ripple rates during offline periods following each block is correlated with the level of offline gains in motor performance on a subject-by-subject and a trial-by-trial basis. These results are very interesting, and could provide direct evidence for the involvement of hippocampal ripples in offline motor sequence learning in humans, which is currently missing in the literature. However, there are major limitations in the methods that make the interpretation of the observed results very difficult, as outlined in my comments below. Without the addition of a proper control condition the overall impact of this study is restricted.*

4.1: *My main concern is regarding the lack of a control condition to account for non-specific effects of motor learning. There are a variety of factors, including motor fatigue, use-dependent practice, task novelty, or cognitive fatigue that may confound the process of sequence learning. As authors mention in* *the Discussion, a necessary control condition is recording the hippocampal ripples, e.g., during performance of a simple sequence (1-2-3-4) over 12 blocks. They acquired some data only in 7 subjects, for only 2 blocks, and without neural recordings. They already report some motor fatigue related to this simple sequence. Without a proper control condition, and examining how the hippocampal ripples relate to similar measures of offline and online gains during this control condition, the impact of the reported results is not clear.*

Thank you for raising this issue. In addition to our behavioral findings from the screening task, we now present findings from new analyses of the MST and from the literature (see suggestion of Reviewer 1, **1.3**), that are also incompatible with a fatigue explanation of online losses (as detailed in response to Reviewer 1, **1.4**). Importantly, we now also present findings from the analysis of the neural data from the screening (control) task in both the manuscript and in **Figure S3**. These findings support the hypothesis that ripples during MST rest breaks underlie motor sequence learning.

Results, lines 142-150:

"We also used screening task data to evaluate whether the increased ripple rate during MST rest breaks was more likely to reflect learning or other processes related to motor performance. We compared the ripple rates during the first two typing periods and the first rest period of the MST with those of the screening task in the same 7 participants (Fig. S3). Ripple rates were similar during typing periods of the MST and screening task (0.09±0.04 vs. 0.08±0.04). In contrast, only during the MST rest break did the ripple rate increase (by 67%), and it was more than double that of the screening task (0.15±0.09 vs. 0.07±0.02). Although not statistically significant in 7 participants, these findings support the hypothesis that ripples during MST rest breaks reflect motor sequence learning."

Discussion, lines 224-236:

"The increased ripple rate during rest compared with typing periods, and its correlation with micro-offline gains, both across participants and across trials, argues for ripples as an important driver of this rapid offline learning. The present findings complement magnetoencephalography findings of hippocampal sequential memory replay during MST rest periods that predicts the magnitude of offline gains[8] and suggest ripples as the underlying mechanism. Importantly, there was no increase in ripple rate during rest compared with typing on a screening task that minimized learning. Although the MST and screening task had similar ripple rates during typing, during the rest break only on the MST did the ripple rate increase and it was more than double the ripple rate during rest on the screening task. Despite having typed more than twice as fast on screening than the MST, ripple rates did not rise during rest. This indicates that the increased ripples and their relationship to offline gains during MST rest breaks are unlikely to reflect recovery from fatigue. These findings instead support the hypothesis that ripples during MST rest breaks underlie motor sequence learning."

Methods, lines 392-394:

"In a control analyses, we examined whether ripple rates differed during MST vs. screening task online and offline periods using paired t-tests in the 7 participants who completed the screening."

4.2: *The lack of a control condition is exacerbated by the proposed definitions of online and offline gains in this study. Their definition of online gain is misleading, as it is vastly confounded by motor/cognitive fatigue during each trial, and the subject's readiness at the beginning of each trial after each rest. In fact, authors show a negative effect of online learning! Also their definition of offline gain is equally confounded by these factors. Establishing any relationship with these within-session measures requires rigorous control conditions to account for non-specific effects of learning.*

As we note above in response to Reviewer 3, **3.6**, and now report in the manuscript, we adopted the standard definitions of micro-online and offline gains. [6-9, 14] When we substitute the average speed of the first three sequences (please see **Table R1** above), which should mitigate readiness effects, and last three sequences in our measures, the results are substantively the same.

Our findings are consistent with previous reports showing negative online gains (i.e., losses) on the MST in young healthy people.(Jacobacci et al., 2020; Mylonas et al., 2024) Despite negative online gains, hippocampal fMRI activation during rest predicts micro-offline gains.(Jacobacci et al., 2020) Since hippocampal activity is not known to correlate with fatigue, this finding is consistent with the interpretation that offline gains reflect motor memory consolidation.

Even during typing periods that show either zero or positive online gains (no change or an increase in speed), fatigue may still have an influence on performance speed that is offset by learning and other factors such as acclimatizing to the task, etc... The relative proportions of the contributions of these non-specific factors to performance will require careful study to disentangle and could not be accomplished in the acute inpatient setting of the present study with participants with temporarily implanted hippocampal electrodes, a limitation that we acknowledge.

Discussion, lines 221-223:

“Although we cannot exclude contributions from non-specific factors related to motor performance (e.g., fatigue due to mental or physical exertion), collectively, these behavioral findings support the hypothesis that micro-offline gains reflect a rapid form of motor memory consolidation.”

And Discussion, lines 251-254:

“It would be useful to manipulate the duration of typing and rest periods and to have more trials of a non-learning control task to disentangle the potential contributions of recovery from fatigue, memory stabilization, memory enhancement, and other factors to micro-offline gains and their relationship to ripples.”

4.3: *The main findings are reported in Figure 3 based on the main effect of ripples between offline and online periods, and their correlation with the gains. However, it seems that both 3A and 3B results are biased by 2 outliers (the 2 red points with high mean ripple rates). It should be clarified if the results still may hold after the removal of these outliers.*

As shown in the **Table R2** below, the results do not substantively change if these two participants are removed from analyses.

We have added the following to Results, lines 122-128:

“Two participants’ mean offline ripple rates could be considered statistical outliers (2.4 and 2.3 standard deviations above the group mean). The data from these participants were retained for analyses since there is no other reason to suspect that they are not valid. In addition, their mean online ripple rates were not outliers (0.7 and 0.5 standard deviations). When their data are removed the offline ripple rate is no longer significantly higher than online ripple rate (t_{14}=1.55, p=.14) but the correlations of offline ripples with offline gains across participants and trials remain significant.”

Table R2.

	17 participants	15 participants
Offline > online gains	t_{16}=3.13, p=.006	t_{14}= 2.48, p=.026
*Gains ~ Ripple Rate * Period + (1 + Ripple Rate | Subject)*		
Ripple Rate	Est.= 2.38, p= .0008	Est.= 2.56, p= .0001
Interaction: RippleRate - Period	Est.= -3.11, p= .0001	Est.= -3.08, p= .00047
OfflineGains ~ Offline Ripple Rate + (1 + Ripple Rate | Subject)		
Offline Ripple Rate	Est.= 2.26, p= .002	Est.= 2.53, p= .0048
OnlineGains ~ Online Ripple Rate + (1 + Ripple Rate | Subject)		
Online Ripple Rate	Est.= -0.73, p= .40	Est.= -0.61, p= .53
Corr: Mean offline ripple rate with offline gains	r=.64, p=.005	r=.68, p=.005
Corr: Mean online ripple rate with mean online gains	r=.006, p=.98	r=.10, p=.72

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