nature portfolio

Peer Review File

Distributed cortical learning through LEC-mediated γ-synchrony

Corresponding Author: Professor Ji-Song Guan

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)
The paper investigates how the RPE signal from the VTA-DA modifies cortical networks during learning. The authors propose that LEC-V-mediated intercortical gamma-synchrony (PLV) is a key neural correlate of VTA-DA reinforcement learning signals. They propose a neural circuit in which VTA-DA activates LEC-V, which subsequently promotes gamma-synchrony to facilitate both learning and memory retention through the activation of cortical latent engrams, as measured in the RSC. Additionally, this study demonstrated, using intracerebral recordings, that gamma-synchrony is also implicated in the processing of RPE in humans. The paper concludes with a bold statement pointing to the LEC-mediated intracortical gamma-synchrony as a required and sufficient mechanism for memory formation.

This paper adopts an elegant approach that combines correlative analysis using freely moving photometry, electrophysiology, and two-photon recordings with causal manipulation of the proposed circuit. However, in several places, the experiments and results are difficult to follow, and the interpretations and conclusions are unclear. Generally, the manuscript is poorly written (the introduction is too brief and critical concepts are not explained, or unclearly explained (ie, theta-gamma phase-locking, inter gamma synchrony)). The discussion is short, and the main results are not put into context. Several points require further clarification, and some additional experiments may be necessary to strengthen the demonstration and fully support the paper's claim.

Major issues:
There is a loose use of the terms theta-coupled synchrony and intercortical gamma synchrony, evident in the introduction and results of Figure 1, which is confusing because these terms refer to different measurements targeting distinct mechanisms. A significant concern is the interpretation of what the authors call "intercortical synchrony." The phase-locking value (PLV) they measure appears to quantify how gamma activity in each brain region is coupled to theta phase, rather than directly testing gamma synchrony between regions. This is a measurement of theta-gated inter-areal γ phase-locking. This suggests that the observed increase reflects stronger theta–gamma cross-frequency coupling rather than true gamma–gamma coherence across RSC, AuD, and M2. If the goal is to demonstrate synchronized communication between cortical regions during reward processing, a more direct approach would be to compute coherence or PLV between gamma oscillations of the different regions themselves. This would determine whether gamma rhythms across regions are phase-aligned with each other, rather than aligned to a common theta reference. As it stands, the data support increased θ-referenced coordination of γ activity, but not necessarily direct γ-band synchrony between regions. I recommend clarifying this in the terminology and interpretation.
Clarifying this distinction and explaining why classical interregional gamma coherence was not employed would strengthen the interpretation of the data, indicating intercortical synchrony in the gamma band, and avoid conflating theta-mediated modulation with genuine intercortical gamma synchrony. The figure should also illustrate how gamma is coupled to theta.
-Also, the band in the illustrations (i.e. Fig. 1b, g, Fig. 4) are in the 20-30 Hz range, which is in the beta band. Why call it gamma?

-Missing optogenetic controls: no controls for optogenetic experiments in fig.1 and fig.2 (laser stimulation in mCherry-injected mice).

-On the role of the VTA-DA network in the PLV effects (Time-lag analysis): Using photometry of VTA-DA neurons, the authors reported a dopaminergic activation 0.18s ahead of cortical PLV increase.
Are the reported time-lags coherent with the estimated number of synapses in the proposed circuit? This needs to be addressed in the discussion.
How did the authors account for the different kinetics (calcium-derived signals vs. electrophysiology) in the analysis? Indeed, a 180ms lag when comparing calcium reporters (extremely slow kinetics), and field recordings (very fast kinetics)?
Why are the PLV changes occur slightly less than 3 seconds after CS onset in Figure 1 but near 4 seconds in Figure 2? Why this difference?

As for the VTA opto stims, there is a huge variability, ranging from -1s to +0.6s, however, when using optogenetic VTA-DA activation, the reported delay was 0.45s, while optogenetic activation of the LEC-5 tends to present an average delay of 0.49s. Those differences in time lags are difficult to understand in the context of the circuit proposed by the study (VTA-DA activated LEC-5 that induces intercortical synchrony). Those inconsistencies may arise from the analysis method used to measure time lags, from a technical issue involving the use of a combination of electrophysiology and calcium reporters, or from an oversimplification of the proposed circuit.
Is a 10Hz sampling rate sufficient to properly detect those time lags (justified by the authors by the time-resolution of PLV analysis that could be accounted for using a sliding-windows based analysis)
Using the gradient function alone may not be the best option to measure the time lag between PLV and VTAda activity. Please provide additional information about the method used (thresholding, duration, or amplitude criterion) or contrast results from the maximum gradient method proposed, compared to alternative methods such as cross-correlation or peak detection.

LEC as a critical nexus for the conversion of RPE signal into cortical gamma PLV:
optogenetic activation of the LEC failed to significantly induce PLV (Extended fig.2), probably because of the low number of animal (given the presence of an obvious outliers), as this demonstration is central to the paper, more mice need to be added for that experiment
optogenetic silencing of the LEC would be a very strong argument if it induce both the abolition of cortical PLV and learning impairments in the task

Double optogenetic experiment in DAT mice * Rbp4 Cre mice (Fig3):
Anatomy: Fig. 3 b displays a very caudal part of the VTA with few TH staining and incomplete colocalisation of TH and mCherry, questioning the specificity of the viral approach, and some mCherry labeling seems to be present in the SNc: Better pictures are needed (showing bilateral transfection at different levels including the injection site and split channel in addition to the merge)
A quantification of the transfection extent in VTA and SN is needed to investigate potential cellular death in the VTA and the very low coexpression of mCherry and TH
In the low-resolution LEC picture in Fig. 3 b, some neurons seem to be mCherry positive
eNphr3.0 potential toxicity: the quantification of TH-positive neurons in the VTA would be instrumental to rule out potential VTA-DA cell death due to eNphr3.0
supplementary optogenetic control group: eNphr3.0 injected mice without laser (or laser during the ITI) is required to rule out a potential toxicity of eNphr3.0 on DA cells
timing of the optogenetic manipulation: authors provide laser stimulation during the delay and response windows (thus targeting reward value encoding and not RPE), why not during the CS as they demonstrated in Fig1 and Fig2 that RPE signals shift backward to the CS with learning? This would be more in line with the paper's claim regarding the role of LEC in mediating RPE signals.
Double Cre mice with AAV9 Cre-dependent virus: AAV9 is known to be potentially both anterograde and retrograde, especially when using Cre-dependent systems. Double optogenetic manipulations in double Cre mice (silencing of VTA fibers in LEC + stim of LEC-5) relied on cre-dependent AAV9 virus in double Cre-mice (Dat*Rbp4 mice); it is possible that the excitatory opsin injected in LEC also retrogradely transfected VTA-DA neurons projecting to the LEC (in this case, the blue laser would coactivate VTA-DA fibers and LEC neurons). The authors should demonstrate that there was no oChIEF expression in VTA-DA or should rely on other constructs than double Cre-mice (DATflp * Rbp4Cre?)
In Fig. 3l, the LEC stimulation had a drastic effect on behavioral performances (asymptotic performances since day 1). Does LEC stimulation affect the licking behavior itself (and not just the percent of rewarded trials as reported in the paper)? Please add that in the supplemental data.
Fig.3j-l: as LEC stimulated mice reach asymptotic performances since day 1 (accelerated learning), was there already a change in PLV at day 1, instead of day 6 as shown in the figure. Also, in Fig. 3j, please provide PLV data for day 1 rather than day 6, as the performance is already maximum in this experiment at day 1, and the paper presumes that PLV changes are instrumental in mediating the RPE signal in the cortex.

Optogenetic LEC activation favoring learning and memory (Fig3m-q): the reported effects on task independent of RPE signaling seems to correspond to the widely known pro-mnesic effect of Entorhinal cortex stimulations so it is unrelated to this RPE-focused paper, and even devaluate the results shown in Fig. 3a-l as this suggests that the rescue of DA silencing by LEC stimulation has potentially nothing to do with RPE and might just be related to a general pro-mnesic effect.

RSC cortex: why focusing on RSC in Fig.4 given the relatively low density of LEC projections to that region (Extended data Fig.4)

Engram cells:

p7-15/17 and Fig.4: neurons from day 6 are retraced on day 3, how was the neurons' registration across days carried on? Examples of registered cells should be provided in Fig. 4.
p7-19/20 and Fig. 4e: “engram cells” classification appears arbitrary: was the proportion of associative cells (on day 3) classified as “recall cells” on day 7 significantly different from the proportion of “other cells” (on day 3) that become “recall cells” on day 7? If not, the Engram cell classification is too arbitrary.
In Fig. 4 h, the comparison between “engram cells” and “non-engram cells” should be restricted to the “recall cells” not classified as “engram cells”; it was maybe the case, but not stated in the manuscript.
p7-33 and Fig. 4i: if PLV changes drive engram cell activation on day 3, why do engram cells precede PLV changes on day 7?
p7-37/38 Fig. 4j: to fully conclude about the role proposed for LEC-mediated gamma sync in engram formation, LEC should be inhibited on day 7, and this should not affect performance nor engram cell activation. This last step of the demonstration would strongly reinforce the paper.

Statistical analysis:
Fig. 2j-l: authors use “one way ANOVA tuckey test”, does it mean “one way ANOVA followed by tuckey postdoc analysis”? If yes, please provide ANOVA values and explain the meaning of the stars. If the stars indicate differences from baseline, the issue is that using normalized data to the baseline value makes the baseline datasets devoid of variability, so ANOVA shouldn’t be used here. Rather, use a t-test comparison with a standard (the zero from the baseline).
Fig. 3f and 3l: repeated measure two-way ANOVA followed by post hoc is required here.

Minimalist introduction:
A clear definition of RPE should be provided in the intro (only appears on page 8, the last section of the results).
A rationale should be given to justify the choice of the cortical regions investigated in terms of PLV and “engram cells” (especially the RSC, given the low LEC projections reported compared to projections to the prefrontal and cingulate cortices for example)
Justify why Rbp4-Cre mice are used.

Minimalist discussion: results are not discussed or compared with relevant literature, despite a very large body of evidence that has already reported a pro-mnesic effect of entorhinal cortex stimulations.

Minor points:

In the intro, they state that their previous work showed that LEC stim and its increase in intercortical gamma reinstated contextual memory. The reference appears not to be from their group, and that's not the conclusion of the cited reference.

Gamma coupling: Why was the gamma band restricted to 20-30Hz? Low gamma is typically defined in the range of 30 to 50Hz, please justify your choice. Their previous 2022 paper used 50Hz. Why is the frequency of gamma different here?

Panel A of Figure 1 is too dense and displays multiple phenomena, making it unclear to this reviewer. This is unhelpful. The same applies to the right panel; it is unclear what the circles at the bottom represent. Where is theta? For panels C and D, the modulation index shows 45-65, but it's significant only at 20-30?

Male and female mice: The Authors stated that both male and female mice were used, so they should indicate for each behavioral group
i) the gender composition and
ii) how the protocols were adapted (if so) for the testing of both genders.

PLV and learning: Fig. 1h relies on linear correlation to demonstrate changes in PLV with learning. This is probably not the best way to statistically analyze change across time, as there is no indication that such a relationship should follow a linear trend (curve fitting might be more appropriate). Use ANOVA rather than being agnostic about the relationship between PLV changes and learning.

VTA-DA activation and PLV changes (Fig. 2d): is reported as a correlation of global averages across days. Cross-correlating the daily time series of VTA-DA with PLV signals would be a stronger argument for a time-locked relationship, and would allow us to correctly estimate the lag between the two signals.

Behavioral results:
Performances in Fig. 1f (very linear) have a very different shape from performances reported in Fig. 3f (plateau from day 4). Moreover, performances are not shown for Figs. 2 and 4. It would be great to display all behavioral performance using the same graph types to facilitate better comparison.
The reported performances are expressed only as the fraction of rewarded trials out of all training trials completed (does it mean that absence of licking during the response period counts as a 0?)… maybe the percent of licking during the response window would be a better measure (in LEC stimulation experiment from fig3 it is possible that the performances of LEC stimulated mice resulted from continuous licking, and not from a better learning)
p6-34 (and Extended data fig.6): When was the running speed recorded? only during the shock (this would be a very short duration, and the speed data would be irrelevant) or during the whole training session (if yes, reported speed ranging from

20 to 80 cm per sec appears very high for mice exploring a conditioning chamber)

Fig.3 fear conditioning: Fig. 3q: a control group (without shock) would be needed to determine if the control mice (mCherry) still have a memory (should be expected at 24hrs) and a control exposition in a non-conditioned environment should be carried out to determine whether LEC stimulated mice have a better memory or just expressed a generalized fear.

Typo:
p4-line 27: “optetrode” -> “optrode”
p5-line 11 “to expedite imaging process” does the author refer to photobleaching? please clarify
p6-line 18-19: Maybe the use of “positive conditioning” instead of reward-tropism and “negative conditioning” instead of punishment-tropism would be better.
p6-line 20: “exploration” instead of “navigation” given the small size of the environment and the type of task.
Fig 4d: X-axis labels are missing for CS-only, Asso, and recall.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)
Overview:
This manuscript presents an impressive array of techniques for both recording and manipulating neural activity in mice, together with intra-cranial EEG data from humans. The paper shows compelling evidence that theta-gamma coupling in neocortex provides a correlate of the dopaminergic RPE. This is an important and novel result and could stand alone as an important and exciting contribution to the field. In addition, the authors claim that the LEC can be defined as a nexus of the interaction between dopaminergic RPEs and other neocortical brain regions. I remain less convinced that the data provided by the authors can support this claim, as articulated in more detail in my comments below. More generally, the paper is extremely dense making it difficult to evaluate – I suggest the authors spend some time decompressing their figures and descriptions thereof.

Major
1. Gamma synchrony as a correlate of RPE: Evidence for gamma as RPE carrier seems strong, although specificity to the gamma oscillation is currently not well evidenced: a) Sometimes the authors seem to refer to theta-gamma synchrony and at other points to gamma synchrony or gamma per se. The definition of ‘gamma’ needs to be clearer throughout.
b) The authors show spectrograms with a very limited frequency range (20-40Hz). Yet other oscillations make obvious candidates, e.g. theta (indeed the authors report increase in theta but do not show it), mid gamma and SWRs, which are all known to support learning and organise mnemonic codes. Can the authors show spectrograms for the full range (say 0-200Hz) and thereby establish whether other oscillations also carry RPE-related information?
c) There is notably some variability in the frequency of peak gamma synchrony that isn’t commented upon. For example, Figure 2g: the VTA opto gives peak around 20Hz, not the peak of around 30Hz which would be expected from Figure 2C.

2. The authors claim that LEC is responsible for transferring the RPE signal from VTADA to other cortical regions (e.g. line 37-38, pg4: ‘the mediating role of LEC in transferring the RPE signal from VTA to the cortex’, and many others), but the authors do not provide clear evidence for spatial or temporal relationships to support this claim. For example, while the imaging of LEC-RSC projections using 2P is impressive it doesn’t speak to a ‘hierarchical cascade, whereby the RPE signal is initially conveyed from the VTADA to LEC, and culminating in an enhancement of intercortical gamma synchrony’. Similarly, Figure 4j claims evidence for a mechanism from LEC to RSC but doesn’t appear to show evidence for this. I raise a number of more specific concerns relating to this point below:
a) Dopaminergic projections are widespread and arguably are sufficient to coordinate RPE across neocortex. For example, weak direct dopaminergic inputs to RSC exist and could account for the reported signals in RSC? There are also alternative candidate pathways such as VTADA dCA1 RSC (where VTA dCA1 is known to support learning and memory). Given a causal manipulation of VTADA dCA1 would likely give similar impairment, how do the authors back their claim that there is something specific about the LEC pathway?
b) To test whether the LEC pathway is necessary can the authors optogenetically silence VTADA inputs received by LEC and then show that in RSC, Aud, M2, the theta-gamma coupling signature of RPE is not observed. Is this what the authors are showing in Figure 3d-e (no brain regions are mentioned for the ephys)? If this is what is shown in Figure 3d-e, then can the authors provide evidence that the laser if not activating axonal boutons from VTADA in RSC, Aud, M2?
c) Again, to support a ‘hierarchical cascade’, convincing temporal relationships are needed but not shown in the current data analyses. For example, line27-30 pg 7 mentions that in RSC PLV preceded the rising time point of calcium activities in RSC engrams. But temporal relationship between ca signals and ephys are non-trivial. Moreover, no temporal relationship with VTADA, LEC and other neocortical regions are reported yet seem critical.
d) The relationship between LEC and RSC seems somewhat unconvincing. Specifically, Figure 2o-p: the response to CS is weak. In fact it looks more like a reward signal (US) response or expected US? The comparison between 1st Assoc and D-US in Figure 2s and 2v would support this view? Does this not undermine the idea that LEC is carrying a RPE signal to RSC. If recorded across days, is there any evidence for a shift from US to CS across days? 2q provides some evidence to suggest less response to US over time but there isn’t evidence for increase in response to CS?
3. Human ECoG data shown in Figure 5. While it is admirable to see cross-species comparisons, I wonder if this data really benefits the rest of the paper. The task is not comparable. Data is then shown for parietal cortex but not frontal and temporal cortices. The reported analyses seems indistinguishable from a surprise or novelty signal as more nuanced RPE

characteristics are not considered. The reported effect seems weak and the exact p value is not reported (Figure 5d). It remains unclear how contacts were selected and whether corrections for multiple comparisons were applied.

Figure specific queries:

1. Figure 3m onwards: need to make clear in the figure legend which viruses are being expressed and when laser is applied.
2. Figure 4b: lower cranial window image needs AP, ML axes marked. Hard to tell which part of RSC this is. Same for Figure 4c.
3. For Figure 4c please show contour map (derived from ca signal average across time) of all imaged cells.
4. For analyses f-I, is there circularity in the definition of engram when comparing to task relevant performance?
5. Figure 3d: what about the mCherry control
6. Figure 3e: which brain regions are being plotted here?
7. Figure 3h: at this resolution it isn't possible to tell if axonal boutons deriving from VTA are apposing the cell bodies labelled with GFP in LEC.
8. Figure 3b: At this resolution it is not possible to see overlap between mCherry and TH. Little explanation in main text or figure that this virus is silencing neural activity and that by mCheery the authors mean an mCherry control that doesn't contain the halorhodopsin?
9. Figure 2C-D: which brain regions do these recordings come from?
10. Figure 2E: what is the p value? What does a negative lag mean given difference in temporal resolution of calcium data and LFP?
11. In Figure 3h it isn't possible to see whether the two viruses are expressed in same location and I couldn't see data for a two laser control using AAV-EF1a-DIO-EGFP and eNpHR3.0 mCherry.

(Remarks on code availability)

Reviewer #3

(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)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Reviewer 1. All my comments were resolved adequately.

1. Theta/gamma terminology and what PLV actually measures is addressed.
2. Calling 20–30 Hz "gamma", is not really gamma, its high-beta, which nearly always starts at 30Hz. Nevertheless, because of consistency with the previous publication, gamma is ok.
3. Missing optogenetic controls, addressed.

4. Time-lag analysis ok.
5. Figure timing inconsistency between old Figs. 1 and 2 ok.

6. Lag variability / lag methodology / 10 Hz resolution ok.

7. Need for a stronger demonstration that LEC is the critical nexus ok
8. Double-opto anatomy / specificity / NpHR toxicity ok.

9. Timing of optogenetic manipulation should match the learned CS period ok
10. AAV9 retrograde-expression concern in the double-Cre rescue design ok
11. Could LEC stimulation simply increase licking? ok.

12. PLV should be shown on day 1 in the rescue experiment ok.
13. LEC stimulation may just have a generic pro-mnesic effect unrelated to RPE ok.
14. Why focus on RSC? ok.

15. Engram-cell registration and classification ok.

16. Why PLV precedes engram activity on day 3 but not day 7, and test LEC inhibition at recall ok.

17. Statistical analyses ok.

18. Introduction lacked a clear RPE definition ok.

19. Rationale for cortical regions and Rbp4-Cre ok.

20. Discussion too minimalist / insufficiently contextualized ok.

21. Minor points 21–25 and 29–30 ok.

22. Minor point 26 (daily cross-correlation of VTA and PLV) ok.

23. Minor points 27–28 (behavior display and performance metric) ok.

(Remarks on code availability)

Reviewer #3

(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)

Dear Editors and Reviewers,

We sincerely appreciate your time and efforts in reviewing our manuscript, "Distributed cortical learning through LEC-mediated γ-synchrony" (NCOMMS-25-84994-T). We acknowledge that your comments and suggestions have been highly valuable in enhancing our manuscript. We have thoroughly revised the manuscript and incorporated new experimental data requested by the reviewers. Figures containing excessive information (Main figures 1-3) have been subdivided to make them easier to review. We are confident that these extensive revisions to both the figures and the text have substantially improved the quality of our manuscript. The changes to the text are highlighted throughout the revised version and are also referenced by page and line number herein. We have systematically addressed the reviewers' overarching concerns as follows:

Reviewer #1 (Remarks to the Author):

The paper investigates how the RPE signal from the VTA-DA modifies cortical networks during learning. The authors propose that LEC-V-mediated intercortical gamma-synchrony (PLV) is a key neural correlate of VTA-DA reinforcement learning signals. They propose a neural circuit in which VTA-DA activates LEC-V, which subsequently promotes gamma-synchrony to facilitate both learning and memory retention through the activation of cortical latent engrams, as measured in the RSC. Additionally, this study demonstrated, using intracerebral recordings, that gamma-synchrony is also implicated in the processing of RPE in humans. The paper concludes with a bold statement pointing to the LEC-mediated intracortical gamma-synchrony as a required and sufficient mechanism for memory formation.

This paper adopts an elegant approach that combines correlative analysis using freely moving photometry, electrophysiology, and two-photon recordings with causal manipulation of the proposed circuit. However, in several places, the experiments and results are difficult to follow, and the interpretations and conclusions are unclear. Generally, the manuscript is poorly written (the introduction is too brief and critical concepts are not explained, or unclearly explained (ie, theta-gamma phase-locking, inter gamma synchrony)). The discussion is short, and the main results are not put into context. Several points require further clarification, and some additional experiments may be necessary to strengthen the demonstration and fully support the paper's claim.

We appreciate the time and effort the reviewer has dedicated to reviewing our manuscript. Thank you very much for your valuable feedback. Due to the volume of issues, we have assigned each one a number to facilitate browsing and location.

Major issues:

1. There is a loose use of the terms theta-coupled synchrony and intercortical gamma synchrony, evident in the introduction and results of Figure 1, which is confusing

because these terms refer to different measurements targeting distinct mechanisms. A significant concern is the interpretation of what the authors call "intercortical synchrony." The phase-locking value (PLV) they measure appears to quantify how gamma activity in each brain region is coupled to theta phase, rather than directly testing gamma synchrony between regions. This is a measurement of theta-gated inter-areal γ phase-locking. This suggests that the observed increase reflects stronger theta–gamma cross-frequency coupling rather than true gamma–gamma coherence across RSC, AuD, and M2. If the goal is to demonstrate synchronized communication between cortical regions during reward processing, a more direct approach would be to compute coherence or PLV between gamma oscillations of the different regions themselves. This would determine whether gamma rhythms across regions are phase-aligned with each other, rather than aligned to a common theta reference. As it stands, the data support increased θ-referenced coordination of γ activity, but not necessarily direct γ-band synchrony between regions. I recommend clarifying this in the terminology and interpretation.

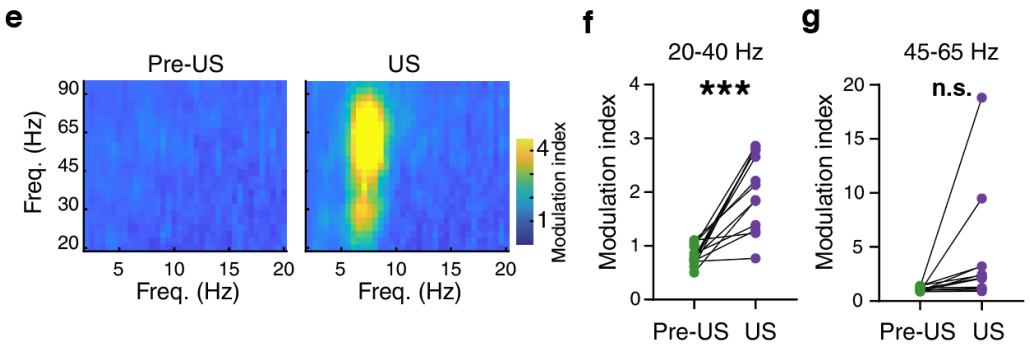
We apologize for the confusing use of the terms "theta-coupled synchrony" and "gamma synchrony" to describe the findings of our study. We agree with the Reviewer that it is necessary to use more parsimonious terms to describe our study's findings and to avoid overinterpretation. This study primarily examines the **interregional gamma band oscillatory synchrony** among three cortical areas (RSC, AuD, and M2) rather than local cross-frequency modulation within a single brain region. In the revised manuscript, we have now resolved this terminology confusion.

Clarifying this distinction and explaining why classical interregional gamma coherence was not employed would strengthen the interpretation of the data, indicating intercortical synchrony in the gamma band, and avoid conflating theta-mediated modulation with genuine intercortical gamma synchrony. The figure should also illustrate how gamma is coupled to theta.

Because our findings indicate that cortical gamma power was not significantly elevated during behavioral training (**Extended Data Fig. 1c**) and that the primary alteration observed was in phase synchronization, we chose not to use the coherence calculation method. We clarify this issue in the text (**Page 3, Lines 12-14**).

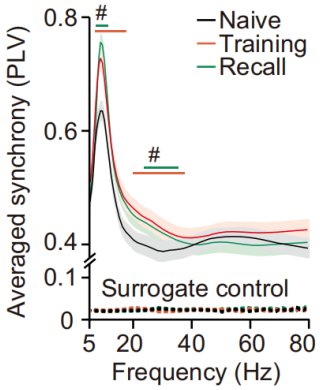
When calculating the modulation index (MI) for the US interval, we initially employed surrogate control (a chance distribution derived from the analysis of surrogate time series that shares statistical properties with the original data, Hurtado et al. 2004) to obtain the normalized values without comparing them to data from the pre-US period. In the revised version, we incorporated this analysis (**see data below**) and found that compared to the pre-US phase, the 20-40 Hz MI in the US interval showed a significant increase (**e**), while the 45-65Hz MI did not (**f**). We noted that two discrete data points elevated the average MI value in this frequency band. However, since the overall result lacked statistical significance, we decided to show only the 20-40 Hz frequency range

in the main figure (**Fig. 1d**) to avoid misinterpretation, placing the 45-65 Hz data in the **Extended Data Fig. 1e-g**.



2. -Also, the band in the illustrations (i.e. Fig. 1b, g, Fig. 4) are in the 20-30 Hz range, which is in the beta band. Why call it gamma?

Spectrograms and data in Fig. 1 revealed significant differences within the 20-30 Hz frequency band, a range generally classified as the high beta frequency band. In order to maintain consistency with the phenomena observed in a previous article that we published (**Ref 17#**, also in the 20–40 Hz range, see data below), we believe that LEC-mediated intercortical synchrony shares similarities with these findings. In addition, in our statistical analysis of all the mouse PLV data, we averaged data from the 20–40 Hz frequency range rather than the 20–30 Hz range.



Comparison of the averaged overall intercortical synchrony in a spatial memory task. Data sourced from **Ref 17#**

Finally, we find some papers also categorize the 25-40 Hz range as the low gamma frequency band:

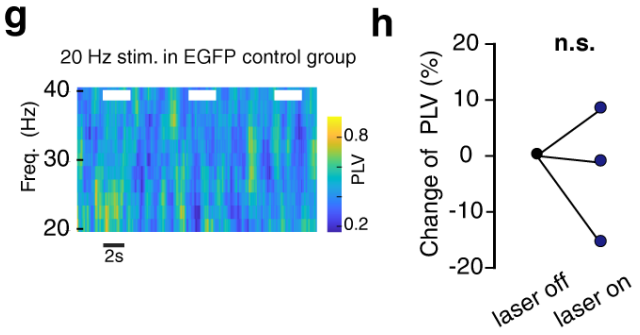
1. Han, Chuanliang et al. *PLoS biology*. 2021 doi:10.1371/journal.pbio.3001466
2. Wang, Yingying et al. *The Journal of Neuroscience*. 2023 doi:10.1523/JNEUROSCI.1294-22.2022

3. Liu, Ling et al. *Science advances*. 2020
doi:10.1126/sciadv.aay4073
4. Araki, Toshihiko et al. *NeuroImage*. 2016
doi:10.1016/j.neuroimage.2016.05.066
5. Bell, L Andrew et al. *The Journal of Physiology*. 2015
doi:10.1113/jphysiol.2014.277814
6. Menon, V et al. *Electroencephalography and clinical neurophysiology*. 1996
doi:10.1016/0013-4694(95)00206-5

In Summary, we believe it is reasonable to define the low gamma band as the 20–40 Hz frequency range in this manuscript.

3. -Missing optogenetic controls: no controls for optogenetic experiments in fig.1 and fig.2 (laser stimulation in mCherry-injected mice).

We observed that the optogenetic experiments mentioned by the Reviewer in fig. 1 and fig. 2 are exclusively shown in fig. 2f-I (**fig. 3f-i now**). This section of the study compared intercortical gamma synchrony levels between laser-on and laser-off conditions in the oChIEF group of mice. We acknowledge the Reviewer's comment and have incorporated an additional experiment with an EGFP control group (**Extended Data Fig. 2g-h**). The results of the control group experiments showed that laser stimulation of the VTA did not enhance intercortical synchrony (average -2.83 ± 6.96 %, n = 3, P = 0.7234, see figure below), thus excluding the possibility of artifact due to laser exposure.



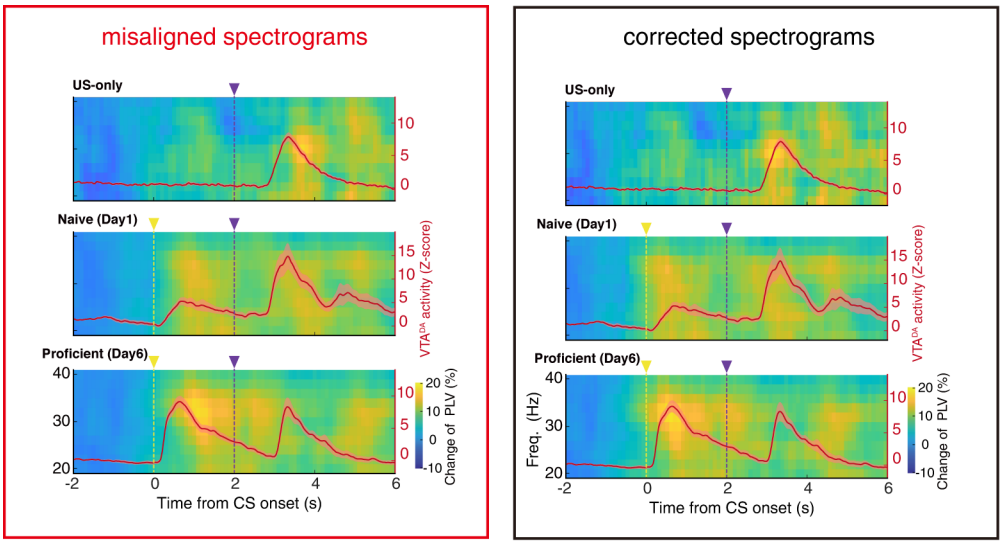
4. -On the role of the VTA-DA network in the PLV effects (Time-lag analysis): Using photometry of VTA-DA neurons, the authors reported a dopaminergic activation 0.18s ahead of cortical PLV increase. Are the reported time-lags coherent with the estimated number of synapses in the proposed circuit? This needs to be addressed in the discussion. How did the authors account for the different kinetics (calcium-derived signals vs. electrophysiology) in the analysis? Indeed, a 180ms lag when comparing calcium reporters (extremely slow kinetics), and field recordings (very fast kinetics)?

Based on previous studies of the VTA-mPFC circuit—which is the target with high density of VTA projections to the cortex—it has been found that the mPFC exhibits a

direct response to VTA neurons within 10 ms (Ellwood, Ian T et al. J Neurosci. 2017 and Lavin A et al. J Neurosci. 2005). The delay we observed in intercortical PLV was over 100 ms, far exceeding the latency of direct response in mPFC. We therefore propose that the observed intercortical synchronization may not result from direct VTA-Cortex projections, but rather be mediated by LEC. As the Reviewer mentioned, calcium signals have slower kinetics compared to electrical signals, and we found that the average rising time of calcium signals with slower temporal resolution is still earlier than that of the faster electrical signals, and then the "true time lag" may be greater than 180 ms, which could still support our conclusion that the VTA activity leads the cortical activity.

5. Why are the PLV changes occur slightly less than 3 seconds after CS onset in Figure 1 but near 4 seconds in Figure 2? Why this difference?

We thank the reviewer for bringing this issue to our attention. Upon comparing our figures with the Reviewer's comment, we surmise that the Reviewer may be referring to the inconsistency in the timing of PLV enhancement emergence in the CS-US training experiments depicted in Fig. 1g and Fig. 2c (**Fig. 3c now**). We observed that the alignment of the PLV data with the concurrent calcium signal in Fig. 3c was incorrect, leading to misaligned time axes in the spectrograms. We have corrected these spectrograms (see figure below). Notably, we confirm that the data in **Fig. 3d-e** are accurate. The average time lag of -0.18 ± 0.04 s between the rising time points of VTA^{DA} calcium signals and those of intercortical PLV within the CS period is consistent with the corrected spectrograms. The averaged time lag between the rising time points of the PLV (20-40 Hz) and Calcium signals is clearly more than -0.18 ± 0.04 s in the original, incorrectly aligned figure. Again, we thank the Reviewer for pointing out our oversight.

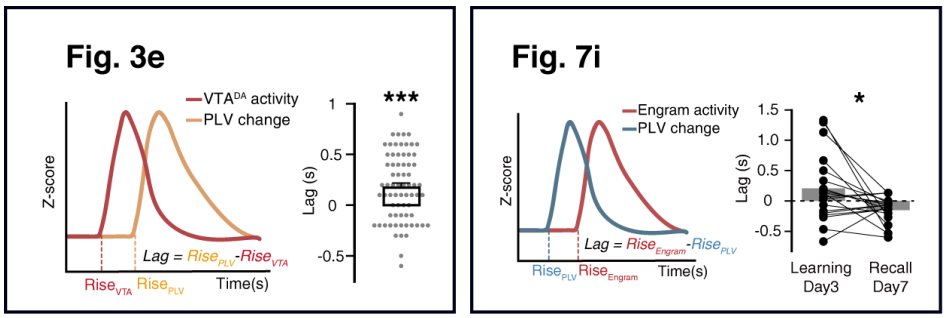


6. As for the VTA opto stims, there is a huge variability, ranging from -1s to +0.6s, however, when using optogenetic VTA-DA activation, the reported delay was 0.45s, while optogenetic activation of the LEC-5 tends to present an average delay of 0.49s. Those differences in time lags are difficult to understand in the context of the circuit proposed by the study (VTA-DA activated LEC-5 that induces intercortical synchrony). Those inconsistencies may arise from the analysis method used to measure time lags, from a technical issue involving the use of a combination of electrophysiology and calcium reporters, or from an oversimplification of the proposed circuit.

We apologize that our positive and negative symbolic representations of the delay time may have caused this misunderstanding. Regarding the time delay results in the right panel of **Fig. 2e (Fig. 3e now)**, we determined the temporal discrepancy between the rising time point of the VTA calcium signal and the elevation of the PLV (as demonstrated in the left panel of **Fig. 3e**). Negative values denote that the VTA^{DA} calcium signal precedes the enhancement of cortical PLV, whereas positive values indicate that the cortical PLV enhancement precedes the VTA calcium signal. The time lags between the two signals indeed fall within the range of -1 to +0.6 s; however, the mean value is **-0.18 ± 0.04** s (**page 4, line 23**), suggesting that the VTA^{DA} calcium signal precedes the cortical PLV enhancement in the majority of trials.

To facilitate comprehension and to standardize the notation, we revise the methodology for calculating delay to Lag = $Rise_{PLV}$ - $Rise_{VTA}$. This modification adjusts the calculated delay to a positive value (**Fig. 3e**). We have also changed the direction of the subtraction calculation in **Fig. 7i**. Please refer to the figure below.

The direction of subtraction for the computed lag has been adjusted to ensure that the lag value is expressed as a positive number.



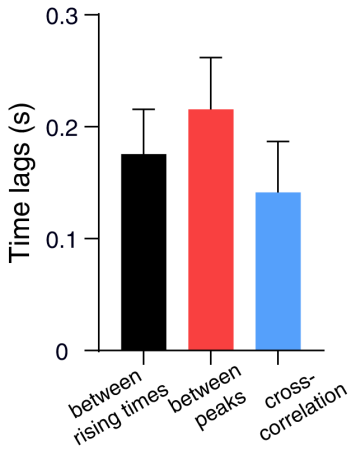
With respect to the results of the VTA optogenetics experiment shown in **Fig. 2i (Fig. 3i now)**, the metric **"delay after laser onset"** was used to illustrate the temporal lag of the PLV enhancement following the first pulse of a 3-second, 20 Hz laser stimulus, where positive values indicate the time difference between the initial laser pulse and the following PLV enhancement. Our results yielded an average delay of 0.45 ± 0.05 s (**page 4, line 26**). This delay surpasses the 0.18 ± 0.04 s latency observed in the previously mentioned fiber photometry recordings, which may be ascribed to the slower dynamic response of the calcium signal. Consequently, the slow dynamics of the

calcium reporter result in closer alignment between the calcium signal and the LFP signals, thereby decreasing the delay. We have added an explanation of the temporal resolution problem across different recording methods to the results section (**page 4, line 27-30**).

The reviewer's comment that **"optogenetic activation of the LEC-5 tends to present an average delay of 0.49 seconds"** may pertain to the data displayed in **Extended Data Fig. 2b-d**. However, this experiment actually aims to optogenetically activate the VTA^{DA} and measure synchronization between the LEC and the other three cortical regions (LEC-Cortex), rather than activating the LEC to measure intercortical synchronization among the three cortices. We think the difference between 0.49 seconds (LEC-Cortex) and 0.45 seconds (RSC, Aud, and M2) can be considered a neural response occurring on the same temporal scale.

Is a 10Hz sampling rate sufficient to properly detect those time lags (justified by the authors by the time-resolution of PLV analysis that could be accounted for using a sliding-windows based analysis)
Using the gradient function alone may not be the best option to measure the time lag between PLV and VTAda activity. Please provide additional information about the method used (thresholding, duration, or amplitude criterion) or contrast results from the maximum gradient method proposed, compared to alternative methods such as cross-correlation or peak detection.

Following the review's suggestion, we conducted a comparison of the time lag differences across various methods (see data below). The statistical analysis indicates that there is no significant difference (one-way ANOVA) among the lag differences obtained through our original rising time detection method (0.18 ± 0.04 s), peak detection method (0.22 ± 0.05 s), or the cross-correlation methods (0.14 ± 0.05 s).



7. LEC as a critical nexus for the conversion of RPE signal into cortical gamma PLV: optogenetic activation of the LEC failed to significantly induce PLV (Extended fig.2), probably because of the low number of animal (given the presence of an obvious

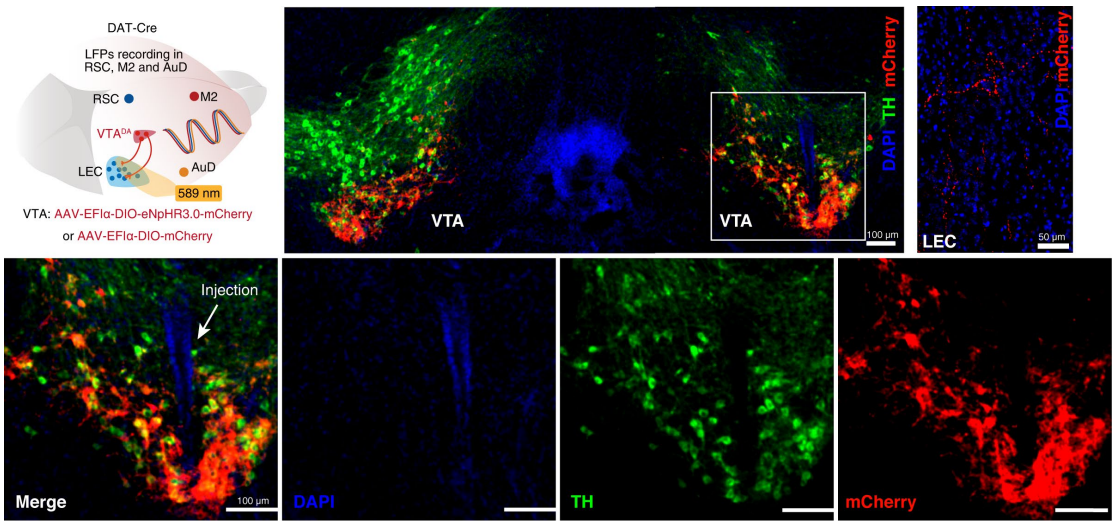
outliers), as this demonstration is central to the paper, more mice need to be added for that experiment. optogenetic silencing of the LEC would be a very strong argument if it induce both the abolition of cortical PLV and learning impairments in the task

It should be noted that in this figure (**Extended Data Fig. 2b-c**), we are analysing LEC-Cortex rather than intercortical synchrony changes after optogenetic activation of the **VTA (instead of LEC)**. Although this study primarily emphasizes intercortical synchronization signals, we also report data from the LEC-Cortex pairs following VTA^{DA} stimulation. Despite the limited sample size, a trend of enhancement remains observable.

In the loss-of-function and rescue experiments in **Fig. 5**, the inhibition of VTA-LEC projections during training effectively suppressed the learning and abolished intercortical synchrony signals. On the other hand, activation of LEC^{5} while simultaneously suppressing upstream VTA^{DA}-LEC projections in DAT-Cre × Rbp4-Cre mice during associative training successfully rescued the intercortical PLV and learning impairments.

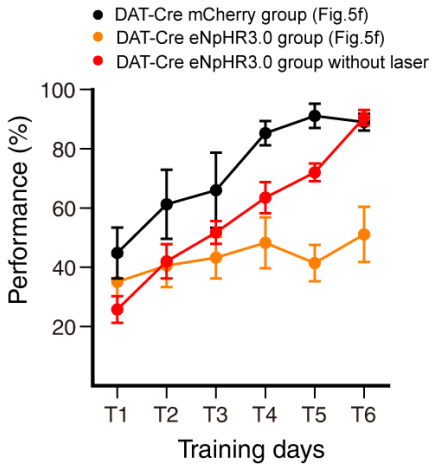
8. Double optogenetic experiment in DAT mice * Rbp4 Cre mice (Fig3):
Anatomy: Fig. 3 b displays a very caudal part of the VTA with few TH staining and incomplete colocalisation of TH and mCherry, questioning the specificity of the viral approach, and some mCherry labeling seems to be present in the SNc:
Better pictures are needed (showing bilateral transfection at different levels including the injection site and split channel in addition to the merge)
A quantification of the transfection extent in VTA and SN is needed to investigate potential cellular death in the VTA and the very low coexpression of mCherry and TH
In the low-resolution LEC picture in Fig. 3 b, some neurons seem to be mCherry positive

We sincerely apologize that the representative images previously selected did not sufficiently depict our viral injections and co-labeling. Based on the reviewer's recommendation, we have refined and incorporated a better image (**see data below**) to demonstrate bilateral VTA virus injections, including a zoomed-in panel and split-channel image to effectively illustrate the co-labeling of virus-expressing cells and TH-positive neurons. We also updated a high-resolution LEC image (**Fig. 5b now**).



eNphr3.0 potential toxicity: the quantification of TH-positive neurons in the VTA would be instrumental to rule out potential VTA-DA cell death due to eNphr3.0
supplementary optogenetic control group: eNphr3.0 injected mice without laser (or laser during the ITI) is required to rule out a potential toxicity of eNphr3.0 on DA cells

We acknowledge the Reviewer's concern that the reduced behavioral performance of mice in the NPHR group may stem from the toxicity of NPHR rather than from optogenetic inhibition of the VTA-LEC loop. To address this, we conducted a control group experiment (injected with NPHR but not exposed to laser stimulation), and observed that these mice still achieved an 80% correctness rate after six days of training (red). This result is significantly different from that of the NPHR-inhibited group described in the original study (yellow).



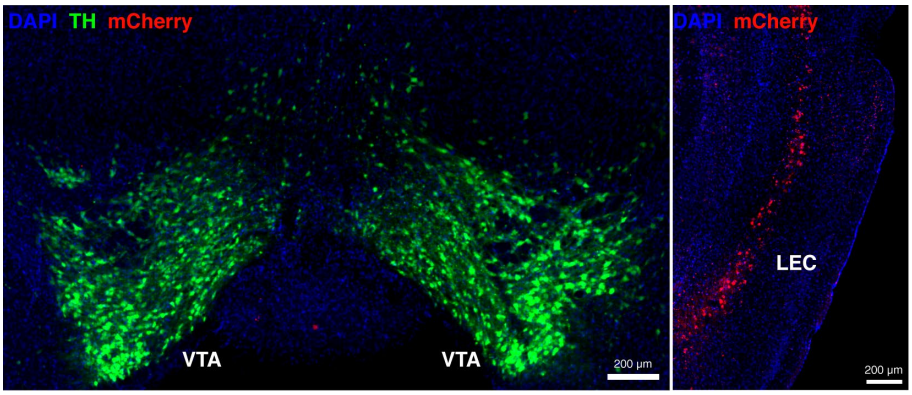
9. timing of the optogenetic manipulation: authors provide laser stimulation during the delay and response windows (thus targeting reward value encoding and not RPE), why not during the CS as they demonstrated in Fig1 and Fig2 that RPE signals shift backward to the CS with learning? This would be more in line with the paper's claim

regarding the role of LEC in mediating RPE signals.

We concur with the reviewer that aligning the timing of the optogenetic stimulus with the observed onset of synchrony may be more appropriate. Our experiments demonstrated that stimulation at T1 was sufficient to promptly enhance the learning performance of mice compared to controls, and no modifications were made to the timing of the optogenetic stimulation during subsequent days of training in this study. Although additional stimulation days did not further improve the behavioral performance of the mice, the findings of this experiment addressed our primary question regarding whether LEC stimulation can rescue the adverse effects of VTA-LEC pathway inhibition. We acknowledge that, although somewhat divergent from our original objectives, adjusting the stimulation timing represents an intriguing and meaningful endeavor to optimize learning efficiency.

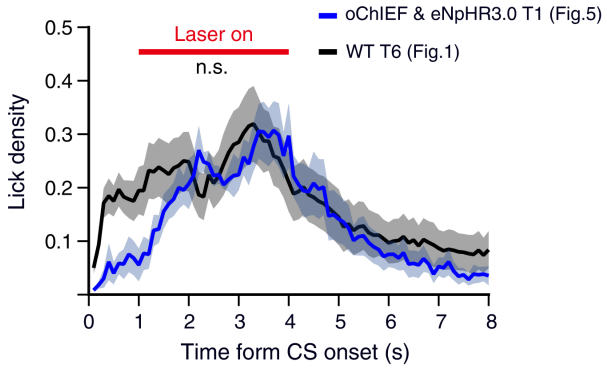
10. Double Cre mice with AAV9 Cre-dependent virus: AAV9 is known to be potentially both anterograde and retrograde, especially when using Cre-dependent systems. Double optogenetic manipulations in double Cre mice (silencing of VTA fibers in LEC + stim of LEC-5) relied on cre-dependent AAV9 virus in double Cre-mice (Dat*Rbp4 mice); it is possible that the excitatory opsin injected in LEC also retrogradely transfected VTA-DA neurons projecting to the LEC (in this case, the blue laser would coactivate VTA-DA fibers and LEC neurons). The authors should demonstrate that there was no oChIEF expression in VTA-DA or should rely on other constructs than double Cre-mice (DATflp * Rbp4Cre?)

We examined viral expression in the VTA of mice injected with the same AAV-DIO-NpHR3.0 virus at the LEC (see image below) and checked that there was no obvious retrograde virus leak.



11. In Fig. 3l, the LEC stimulation had a drastic effect on behavioral performances (asymptotic performances since day 1). Does LEC stimulation affect the licking behavior itself (and not just the percent of rewarded trials as reported in the paper)? Please add that in the supplemental data.

In this study, CS-US training performance is determined by the ratio of trials in which the response window was hit to the total number of trials. If the mouse did not lick in the response window of the current trial, the trial was recorded as a miss. The question of whether stimulation of the LEC inherently promotes water-licking behavior in mice, rather than being a learned response, has significant implications for the interpretation of our experimental results. To address this, we reexamined the probability of water licking in mice receiving LEC stimulation on training day1 (fast learning) versus that of WT mice on day 6 (exhibiting equivalent behavioral performance levels). LEC stimulation was administered during both the delay and response intervals (1-4 s, red bar); however, the data indicated that LEC stimulation did not significantly improve the licking behavior of mice during the laser-on period. Therefore, it can be concluded that LEC stimulation alone was insufficient to induce an enhancement in water licking behavior in mice.



12. Fig.3j-l: as LEC stimulated mice reach asymptotic performances since day 1 (accelerated learning), was there already a change in PLV at day 1, instead of day 6 as shown in the figure. Also, in Fig. 3j, please provide PLV data for day 1 rather than day 6, as the performance is already maximum in this experiment at day 1, and the paper presumes that PLV changes are instrumental in mediating the RPE signal in the cortex.

We concur with the reviewer's suggestion to modify the data presentation from day 6 to day 1, as depicted and analyzed in **Fig. 5j-k and Extended Data Fig. 6b**.

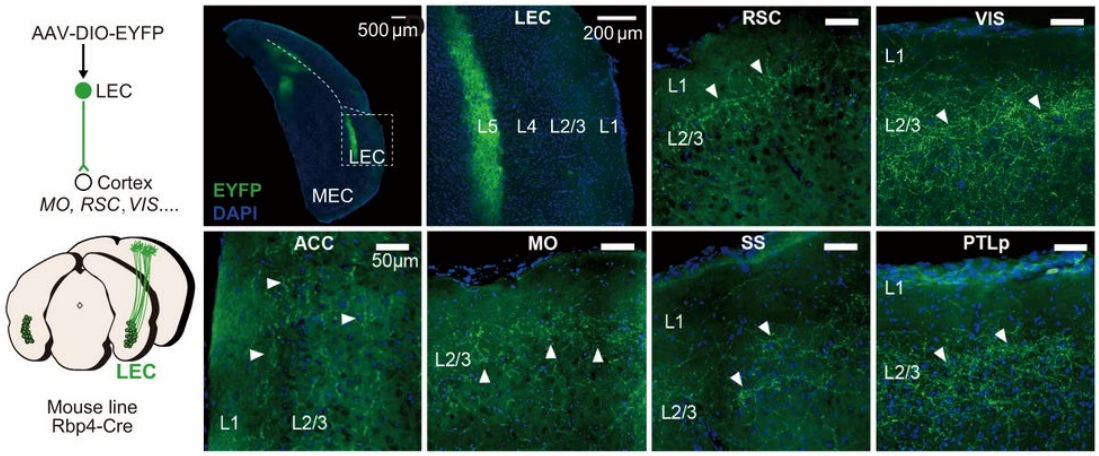
13. Optogenetic LEC activation favoring learning and memory (Fig3m-q): the reported effects on task independent of RPE signaling seems to correspond to the widely known pro-mnesic effect of Entorhinal cortex stimulations so it is unrelated to this RPE-focused paper, and even devaluate the results shown in Fig. 3a-l as this suggests that the rescue of DA silencing by LEC stimulation has potentially nothing to do with RPE and might just be related to a general pro-mnesic effect.

In this study, we aim to investigate how reinforcement learning signals contribute to the formation of long-term memory. Our data showed the important role of LEC^5 in both reinforcement learning and long-term memory storage. These findings suggest that LEC^5-mediated intercortical γ-synchrony serves as the nexus connecting reinforcement learning with the formation of cortical memory networks.

Furthermore, a positive reward prediction-error (+RPE) occurs when the actual reward is better than expected (i.e. rewards in the CPP experiment), while a negative reward prediction-error (-RPE) happens when the actual reward is worse than expected (i.e. foot shock in CFC experiment). Previous research examining the interaction between reward prediction-error and memory has unveiled two conflicting findings: one is the signed reward prediction-error (SRPE) effect, while the other is the unsigned reward prediction error (URPE) or surprise prediction-error effect. The SRPE model posits that memory is regulated by the direction of deviation, with positive RPE being most conducive to memory. In contrast, the URPE model emphasises the absolute magnitude of deviation, positing that memory encoding is enhanced whenever outcomes deviate significantly from expectations, irrespective of the outcome's valence (Rouhani, Nina, and Yael Niv. "Signed and unsigned reward prediction errors dynamically enhance learning and memory." eLife). Our research indicates that stimulating LEC not only enhances spatial memory regarding reward locations but also strengthens context-dependent fear memories. These findings further support the URPE model.

14. RSC cortex: why focusing on RSC in Fig.4 given the relatively low density of LEC projections to that region (Extended data Fig.4)

We acknowledge that the results of anterograde tracing indicate a limited number of neurons downstream of LEC in the RSC brain region; however, this may be attributable to the inefficiency of the AAV1 virus. Our previous study (**Ref 17#**) identified a substantial number of LEC projection fibers within RSC regions by examining LEC projection axons (see figure below). Additionally, a mapping study has demonstrated that the axonal projection intensity of the LEC5 in the retrosplenial region was the highest among the neocortex (*Lu, J., Zhang, Z., Yin, X. et al. An entorhinal-visual cortical circuit regulates depression-like behaviors. Mol Psychiatry 27, 3807–3820 (2022).* https://doi.org/10.1038/s41380-022-01540-8).



Efferent axons from L5 neurons of LEC were detected in a wide range of cortical areas in L2/3, including, MO, RSC, VIS, SS, PTLp (Posterior parietal cortex) and ACC (Anterior cingulate cortex). Data sourced from **Ref 17#.**

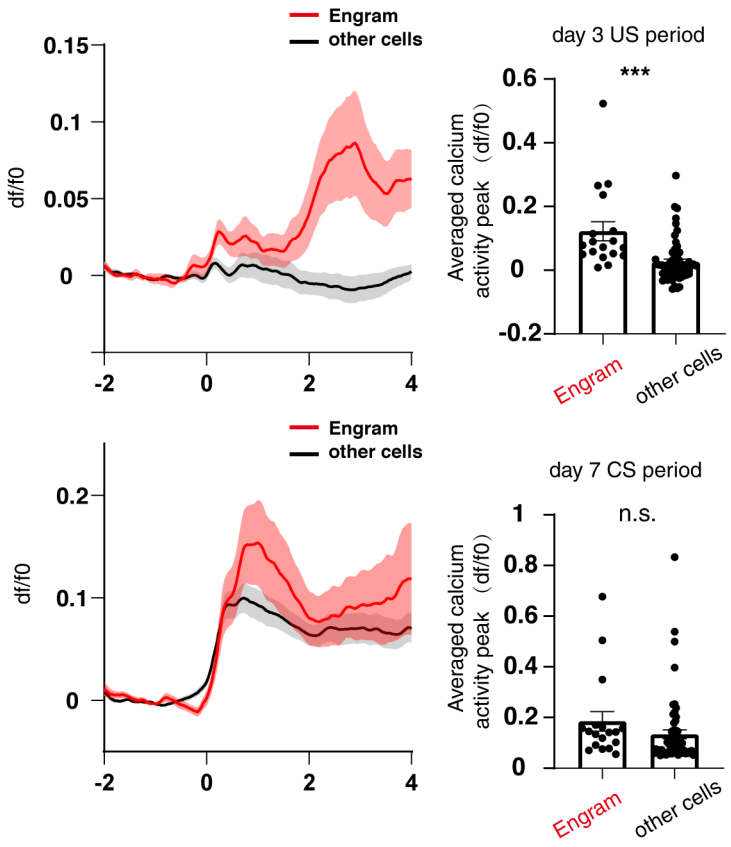
15. Engram cells:
p7-15/17 and Fig.4: neurons from day 6 are retraced on day 3, how was the neurons' registration across days carried on? Examples of registered cells should be provided in Fig. 4.

2P data processing, which included picture alignment and data acquisition, was conducted using custom-written semi-automatic MATLAB codes (**Ref 55#**). The tag channel, indicating the start of sensory stimulation, was first separated from the imaging data. Picture alignment was then performed on the GCaMP6f channel to correct for brain motion caused by animal movement. Each frame of the first baseline trial was aligned to the first frame based on the maximum correlation coefficient, with a maximum shift of 50 pixels in both the x and y axis, between the translated images and the reference image. All frame of the first baseline trial were then averaged to create a high-quality reference image. Subsequent trials were aligned with this reference image using the same algorithm. To align images captured on different days, both translation and rotation were performed to correct for brain motion and head fixation angle deviation. To extract the fluorescence signals from each neuron, we manually labeled the GCaMP6f+ neurons using ImageJ. We used the mean, maximum intensity and standard deviation of all frames in a trial to determine region of interest (ROI) for each GCaMP6f+ neuron across days. The fluorescence signal of each neuron was calculated as the mean of all pixels within its ROI.

Additionally, as Reviewer 2# suggested, we have updated this panel to include multiple representative calcium transients (**Fig. 7b-c**).

p7-19/20 and Fig. 4e: "engram cells" classification appears arbitrary: was the proportion of associative cells (on day 3) classified as "recall cells" on day 7 significantly different from the proportion of "other cells" (on day 3) that become "recall cells" on day 7? If not, the Engram cell classification is too arbitrary.

Thanks to the reviewer's suggestion, we overlooked the analysis for this particular group of cells. By comparing the average calcium activity data of these two groups of neurons on day 7, we observed that the activity of neurons identified as recall cells in the associative group was not significantly different from that of the "other cells" group on day 3. **The key criterion in our study for distinguishing an engram is whether it exhibits strong calcium activity on both day 3 and day 7.** In contrast, the averaged calcium activity of the "other cells" group was not significant on day 3 (see figure below).



In Fig. 4 h, the comparison between “engram cells” and “non-engram cells” should be restricted to the “recall cells” not classified as “engram cells”; it was maybe the case, but not stated in the manuscript.

Again, the key criterion in our study for distinguishing an engram is whether it exhibits strong calcium activity on both day 3 and day 7. All cells active on day 7 are classified as recall cells, and only a small number of those that were also active on day 3 are referred to as engrams. Here, we are comparing the 18 engram cells with all the remaining cells.

16. p7-33 and Fig. 4i: if PLV changes drive engram cell activation on day 3, why do engram cells precede PLV changes on day 7?
p7-37/38 Fig. 4j: to fully conclude about the role proposed for LEC-mediated gamma sync in engram formation, LEC should be inhibited on day 7, and this should not affect performance nor engram cell activation. This last step of the demonstration would strongly reinforce the paper.

Given the observation that the time lag decreases during the recall phase, we infer that once the memory Engram network is formed under the modulation of intercortical PLV signals, the reactivation of the Engrams becomes the primary factor in memory retrieval, while the importance of LEC-mediated intercortical synchrony correspondingly decreases.

We fully agree that the proposed experiment would further improve our work. Due to the complexity of the surgery and 2P imaging experiments, we injected hM4D in bilateral LEC and GCaMP6f in RSC in a batch of Rbp4-Cre mice (n=6, **Extended Data Fig. 9**). In accordance with the reviewer's recommendation, we injected CNO intraperitoneally into the animals on day 8 to inhibit LEC activity and injected PBS on day 9. Consistent with our model (**Fig. 7j**), our findings indicate that the inhibition of the LEC did not influence the activity of Engram cells nor impair the behavioral performance of the mice.

17. Statistical analysis:
Fig. 2j-l: authors use "one way ANOVA tuckey test", does it mean "one way ANOVA followed by tuckey postdoc analysis"? If yes, please provide ANOVA values and explain the meaning of the stars. If the stars indicate differences from baseline, the issue is that using normalized data to the baseline value makes the baseline datasets devoid of variability, so ANOVA shouldn't be used here. Rather, use a t-test comparison with a standard (the zero from the baseline).

We concur with the Review's recommendation to substitute the current statistical method in **Fig. 2l** (**Fig. 4c now**) with a paired t-test comparison against the normalized baseline. The new statistics have been updated in the figure and summary of statistical tests in **Extended Table 1**.

Fig. 3f and 3l: repeated measure two-way ANOVA followed by post hoc is required here.

We reanalyzed these results using a two-way ANOVA with Sidak's post hoc multiple comparisons test. The updated statistics are shown in the figure (**Fig. 5f and 5l now**) and in the summary of statistical tests in **Extended Table 1**.

18. Minimalist introduction:
A clear definition of RPE should be provided in the intro (only appears on page 8, the last section of the results).

We acknowledge that the concept of RPE was insufficiently defined in the introduction, and we have now improved this in the main text (**Page 2, Lines 5-8**).

19. A rationale should be given to justify the choice of the cortical regions investigated in terms of PLV and "engram cells" (especially the RSC, given the low LEC projections reported compared to projections to the prefrontal and cingulate cortices for example)
Justify why Rbp4-Cre mice are used.

Regarding the selection of RSC, we have already responded to a similar preceding question (**14# issue**). We selected Rbp4-cre mice because of the selective expression of

Rbp4 in cortical layer 5 neurons, as described in the manuscript (**P4, Lines 33**).

20. Minimalist discussion: results are not discussed or compared with relevant literature, despite a very large body of evidence that has already reported a pro-mnesic effect of entorhinal cortex stimulations.

Thanks to your suggestion, we've strengthened the discussion of our results.

Minor points:

21. In the intro, they state that their previous work showed that LEC stim and its increase in intercortical gamma reinstated contextual memory. The reference appears not to be from their group, and that's not the conclusion of the cited reference.

We confirmed that the results cited in the introduction (**p2, line 20**) are indeed from a previously published paper from our lab (**Ref. #17**: Luo, W., Yun, D., Hu, Y., Tian, M., Yang, J., Xu, Y., Tang, Y., Zhan, Y., Xie, H., & Guan, J. S. (2022). Acquiring new memories in neocortex of hippocampal-lesioned mice. Nature communications, 13(1), 1601. https://doi.org/10.1038/s41467-022-29208-5).

22. Gamma coupling: Why was the gamma band restricted to 20-30Hz? Low gamma is typically defined in the range of 30 to 50Hz, please justify your choice. Their previous 2022 paper used 50Hz. Why is the frequency of gamma different here?

Regarding the definition of the low gamma band, we have already responded to a similar preceding question (**2# issue**). Our 2022 paper (**Ref 17#**) used 20-40 Hz instead of 50 Hz.

23. Panel A of Figure 1 is too dense and displays multiple phenomena, making it unclear to this reviewer. This is unhelpful. The same applies to the right panel; it is unclear what the circles at the bottom represent. Where is theta? For panels C and D, the modulation index shows 45-65, but it's significant only at 20-30?

We agree that certain figures contain excessive information, and in the revised version, these figures have been subdivided to make them easier to review.

In the right panel of **Fig.1a**, we illustrate representative low-gamma band (20–40 Hz, dark lines) phase extraction from the LFP raw traces (tinted lines) in three brain regions. Phase locking value (PLV) is calculated by the difference ($\Delta\varphi$) between the instantaneous phases of the signals from these brain regions. The t1 and t2 time points are chosen from two consecutive RSC γ oscillation troughs, and the t3 and t4 time points are similarly chosen from two consecutive troughs in another time segment. We plotted the phase angles of these three brain regions at each moment on the circle below

(blue arrows show the phase of RSC, red arrows show the phase of M2, and yellow arrows show the phase of AuD). Using the phase difference changes between the RSC and M2 regions ($\Delta\varphi_{M2\text{-}RSC}$) as an example, the phase difference was more stable during the t3-t4 intervals compared to the t1-t2 interval, indicating increased inter-regional synchrony during this period. We have added an explanation of the schematic diagram in the figure legend.

In the revised version, we incorporated this analysis and found that, compared with the pre-US phase, 20-40 Hz MI during the US interval increased significantly, whereas 45-65Hz MI did not. We included the revised figure and analysis in the main **Fig. 1d,** and **Extended Data Fig. 1e-g**.

As Reviewer 2# pointed out, we have extended the PLV analysis to 200 Hz (**Fig. 1c**) and added spectrograms for both the theta band and the high gamma band (**Extended Data Fig. 1d**). No significant changes were observed in the high-gamma band. Furthermore, during the learning task, theta band synchronization increased in both the CS and US phases (**Extended Data Fig. 1i**). Cross-correlation with the water-licking curves showed that theta band synchronization might be linked to water-licking actions rather than carrying the RPE information (**Extended Data Fig. 1j-k**).

24. Male and female mice: The Authors stated that both male and female mice were used, so they should indicate for each behavioral group
i) the gender composition and
ii) how the protocols were adapted (if so) for the testing of both genders.

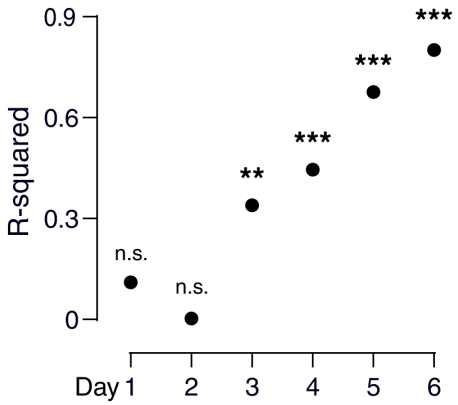
We have disclosed the full gender composition of the mice in **Extended Table 5**. In the Materials and Methods section, we stated that mice of different genders are not treated differently across experimental methods.

25. PLV and learning: Fig. 1h relies on linear correlation to demonstrate changes in PLV with learning. This is probably not the best way to statistically analyze change across time, as there is no indication that such a relationship should follow a linear trend (curve fitting might be more appropriate). Use ANOVA rather than being agnostic about the relationship between PLV changes and learning.

The statistical method used in **Fig.1h** was revised to one-way ANOVA multiple comparisons, and the corresponding statistical values have been updated in the text, the figure legend, and the statistical summary in **Extended Table 1**.

26. VTA-DA activation and PLV changes (Fig. 2d): is reported as a correlation of global averages across days. Cross-correlating the daily time series of VTA-DA with PLV signals would be a stronger argument for a time-locked relationship, and would allow us to correctly estimate the lag between the two signals.

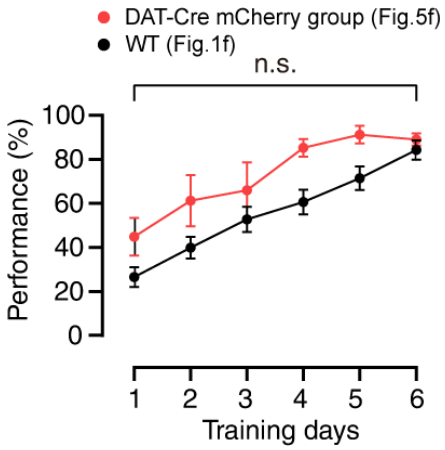
In Fig. 2d (**Fig. 3d now**), we performed a linear regression of the daily PLV enhancement and the magnitude of calcium activity. In accordance with the recommendation, a daily cross-correlation analysis was performed between the calcium signal and PLV alterations during the CS period. The results, illustrated in the figure below, demonstrate that the correlation coefficients between these signals gradually increased as training advanced. The cross-correlation between VTA^{DA} calcium activity and PLV changes from day 3 to day 6 was statistically significant, with the highest correlation coefficient observed on day 6 ($r^2 = 0.80$). The time lag on day 6 calculated by this method was 0.14 ± 0.05 seconds.



Behavioral results:

27. Performances in Fig. 1f (very linear) have a very different shape from performances reported in Fig. 3f (plateau from day 4). Moreover, performances are not shown for Figs. 2 and 4. It would be great to display all behavioral performance using the same graph types to facilitate better comparison.

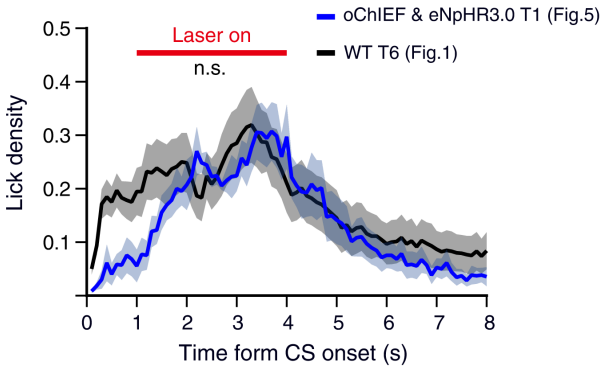
We compared the behavioral performance of control and WT mice across two experimental batches, observing no statistically significant difference (**see data below**). Given that the experimental conditions were consistent, the observed disparities in means may be attributed to variations between different experimental batches.



We apologize for the omission of reporting behavioral data for the optrode experiment and RSC 2P recordings; the relevant figures have been refined in the **Extended Data Fig. 3b** and **Extended Data Fig. 7g**, respectively.

28. The reported performances are expressed only as the fraction of rewarded trials out of all training trials completed (does it mean that absence of licking during the response period counts as a 0?)… maybe the percent of licking during the response window would be a better measure (in LEC stimulation experiment from fig3 it is possible that the performances of LEC stimulated mice resulted from continuous licking, and not from a better learning)

We believe this concern is related to the **11# issue** mentioned earlier. The question of whether stimulation of the LEC inherently promotes water-licking behavior in mice, rather than being a learned response, has significant implications for the interpretation of our experimental results. To address this, we reexamined the probability of water licking in mice receiving LEC stimulation on training day 1 (fast learning) versus that of WT mice on day 6 (exhibiting equivalent behavioral performance levels). LEC stimulation was administered during both the delay and response intervals (1-4 s, red bar); however, the data indicated that LEC stimulation did not significantly improve the licking behavior of mice during the laser-on period. Therefore, it can be concluded that LEC stimulation alone was insufficient to induce an enhancement in water licking behavior in mice.



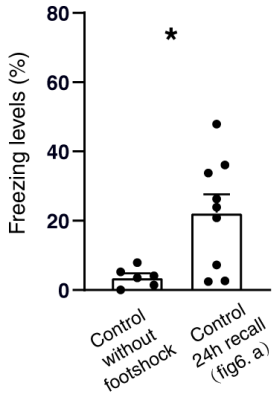
29. p6-34 (and Extended data fig.6): When was the running speed recorded? only during the shock (this would be a very short duration, and the speed data would be irrelevant) or during the whole training session (if yes, reported speed ranging from 20 to 80 cm per sec appears very high for mice exploring a conditioning chamber)

We apologize for not defining running speed more precisely. Yes, we are analyzing the mouse's instantaneous speed after receiving a foot shock. We have added a further description in the corresponding figure and legend. Our purpose in showing this data was to demonstrate, to some extent, that the two groups of mice exhibited comparable pain responses following the foot shock.

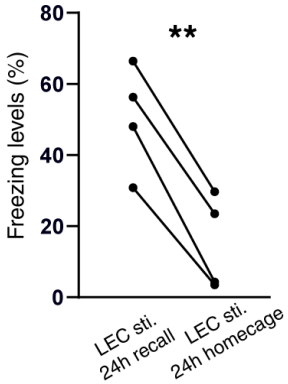
30. Fig.3 fear conditioning: Fig. 3q: a control group (without shock) would be needed

to determine if the control mice (mCherry) still have a memory (should be expected at 24hrs), and a control exposition in a non-conditioned environment should be carried out to determine whether LEC stimulated mice have a better memory or just expressed a generalized fear.

We acknowledge the reviewer's concerns and have conducted two additional experiments in accordance with the recommendations. The data from the first control experiment indicated that mice (n = 6) not exposed to foot shock showed freezing levels significantly lower than those observed in the mCherry mice (which received foot shock) 24 hours later in the training context (refer to data in **Fig. 6e**). $P = 0.0146$, t = 2.816, unpaired t-test. The results showed that the mCherry mice, despite having lower freezing levels than the LEC^5 sti. mice, still had a fear memory of the CFC training context (see figure below).



In the second experiment, we stimulated LEC^5 during CFC training in four new oChIEF-expressing mice and assessed freezing levels 24 hours after in both the CFC training context and the homecage. The results indicated that freezing levels in the CFC training context were markedly elevated compared with those observed in the homecage ($P = 0.002$, t = 10.19, paired t-test), thereby ruling out the possibility of a generalized fear response (see figure below).



Typo:

p4-line 27: “optetrode” -> “optrode”

We have corrected this typo.

p5-line 11 “to expedite imaging process” does the author refer to photobleaching? please clarify

We have clarified this with a more precise statement, “**to shorten imaging durations and prevent photobleaching**” (**Page 5, Lines 20-21**).

p6-line 18-19: Maybe the use of “positive conditioning” instead of reward-tropism and “negative conditioning” instead of punishment-tropism would be better.

We have revised both terms.

p6-line 20: “exploration” instead of “navigation” given the small size of the environment and the type of task.

We have revised this word.

Fig 4d: X-axis labels are missing for CS-only, Asso, and recall.

We appreciate your suggestions. The datasets presented side-by-side in Fig. 4d (**Fig. 7d now**) utilize a consistent X-axis coordinate scale. To conserve space, we initially provided labels on only one set of data. However, recognizing that this may pose challenges for reviewers and future readers, we have now included comprehensive X-axis coordinate scales for each group.

Reviewer #2 (Remarks to the Author):

Overview:
This manuscript presents an impressive array of techniques for both recording and manipulating neural activity in mice, together with intra-cranial EEG data from humans. The paper shows compelling evidence that theta-gamma coupling in neocortex provides a correlate of the dopaminergic RPE. This is an important and novel result and could stand alone as an important and exciting contribution to the field. In addition, the authors claim that the LEC can be defined as a nexus of the interaction between dopaminergic RPEs and other neocortical brain regions. I remain less convinced that the data provided by the authors can support this claim, as articulated in more detail in my comments below. More generally, the paper is extremely dense making it difficult to evaluate – I suggest the authors spend some time decompressing their figures and descriptions thereof.

We sincerely appreciate your support and feedback. We agree that certain figures contain excessive information, and in the revised version, these figures have been subdivided to facilitate an easier review.

Major
1. Gamma synchrony as a correlate of RPE: Evidence for gamma as RPE carrier seems strong, although specificity to the gamma oscillation is currently not well evidenced: a) Sometimes the authors seem to refer to theta-gamma synchrony and at other points to gamma synchrony or gamma per se. The definition of 'gamma' needs to be clearer throughout.

Both reviewers raised this issue. We apologize for the confusing use of the terms "theta-coupled synchrony" and "gamma synchrony" to describe the findings of our study. This study primarily examines the **interregional oscillatory synchrony** among three cortical areas (RSC, AuD, and M2) rather than local cross-frequency modulation within a single brain region. In the revised manuscript, we have now resolved this terminology confusion.

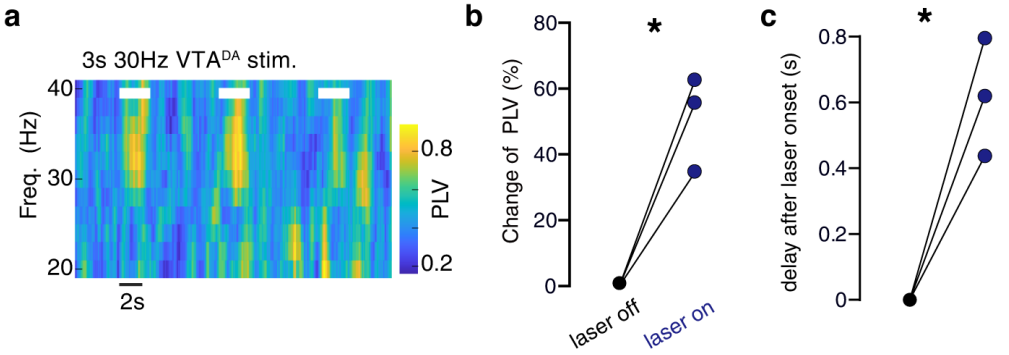
b) The authors show spectrograms with a very limited frequency range (20-40Hz). Yet other oscillations make obvious candidates, e.g. theta (indeed the authors report increase in theta but do not show it), mid gamma and SWRs, which are all known to support learning and organise mnemonic codes. Can the authors show spectrograms for the full range (say 0-200Hz) and thereby establish whether other oscillations also carry RPE-related information?

We thank the reviewer for raising this issue. We have extended the PLV analysis to 200 Hz (**Fig. 1c**) and added spectrograms for both the theta band and the high gamma band (**Extended Data Fig. 1d**). No significant changes were observed in the high-gamma

band. Furthermore, during the learning task, theta band synchronization increased in both the CS and US phases (**Extended Data Fig. 1i**). Cross-correlation with the water-licking curves showed that theta band synchronization might be linked to water-licking actions rather than carrying the RPE information (**Extended Data Fig. 1j-k**).

c) There is notably some variability in the frequency of peak gamma synchrony that isn't commented upon. For example, Figure 2g: the VTA opto gives peak around 20Hz, not the peak of around 30Hz which would be expected from Figure 2C.

We did indeed observe that the synchrony frequency in the fiberphotometry experiments was slightly higher than that shown in Figure 1. Throughout the paper, we defined the 20–40 Hz frequency range as the low-gamma band for all mouse experiments and performed statistical analyses using data from this frequency band. When determining the light pulse frequency for optogenetic stimulation of the VTA^{DA}, we referenced the phasic firing rate of VTA dopamine neurons (**20–30 Hz, Ref 4#, Page 4 Lines 24-25**). We found that stimulating VTA^{DA} at 20 Hz induced 20 Hz synchronization in the cortex. In the revision, we included a set of experiments using 30 Hz optical stimulation of VTA^{DA}. The results showed that optogenetic stimulation of the unilateral VTA^{DA} at 30 Hz also elicited a consistent augmentation (average 50.64 ± 8.38 %, n = 3, see figure below) in ipsilateral intercortical γ-synchrony following a brief delay (0.61 ± 0.10 s, see figure below).



2. The authors claim that LEC is responsible for transferring the RPE signal from VTADA to other cortical regions (e.g. line 37-38, pg4: 'the mediating role of LEC in transferring the RPE signal from VTA to the cortex', and many others), but the authors do not provide clear evidence for spatial or temporal relationships to support this claim. For example, while the imaging of LEC-RSC projections using 2P is impressive it doesn't speak to a 'hierarchical cascade, whereby the RPE signal is initially conveyed from the VTADA to LEC, and culminating in an enhancement of intercortical gamma synchrony'. Similarly, Figure 4j claims evidence for a mechanism from LEC to RSC but doesn't appear to show evidence for this. I raise a number of more specific concerns relating to this point below:

a) Dopaminergic projections are widespread and arguably are sufficient to coordinate

RPE across neocortex. For example, weak direct dopaminergic inputs to RSC exist and could account for the reported signals in RSC? There are also alternative candidate pathways such as VTADA◊ dCA1 ◊ RSC (where VTA◊ dCA1 is known to support learning and memory). Given a causal manipulation of VTADA ◊ dCA1 would likely give similar impairment, how do the authors back their claim that there is something specific about the LEC pathway?

The present study does not preclude the involvement of circuits connecting the VTA to the hippocampus, or direct VTA projections to the cortex, in the processes of reinforcement learning and memory storage. Given the circuit-specific approach consistently employed in our circuit manipulation studies (optical fibers implanted exclusively in the LEC region without involving other cortical areas), we believe our manipulations exclusively altered the VTA-LEC circuitry.

b) To test whether the LEC pathway is necessary can the authors optogenetically silence VTADA inputs received by LEC and then show that in RSC, Aud, M2, the theta-gamma coupling signature of RPE is not observed. Is this what the authors are showing in Figure 3d-e (no brain regions are mentioned for the ephys)? If this is what is shown in Figure 3d-e, then can the authors provide evidence that the laser if not activating axonal boutons from VTADA in RSC, Aud, M2?

Yes, we indeed optogenetically silence VTA^{DA}-LEC projections and then observe that in RSC, Aud, M2, the intercortical synchrony signature of RPE is not observed (**Fig. 5d-e now**). Again, given the circuit-specific approach consistently employed in our circuit manipulation studies (optical fibers implanted exclusively in the LEC region without involving other cortical areas), we believe our manipulations exclusively altered the VTA-LEC circuitry, without activating axonal boutons from VTA^{DA} in RSC, Aud, and M2.

Furthermore, we think evidence supporting the notion that the VTA regulates intercortical synchronization via the LEC rather than directly can be found in the optogenetics stimulation experiments (**Fig. 3f-I now**), where we measured a latency of over 100 ms for the PLV enhancement following optical stimulation of the VTA—significantly longer than the latency previously observed for the direct VTA-Cortex pathway (< 10 ms, Ellwood, Ian T et al. J Neurosci. 2017 and Lavin A et al. J Neurosci. 2005).

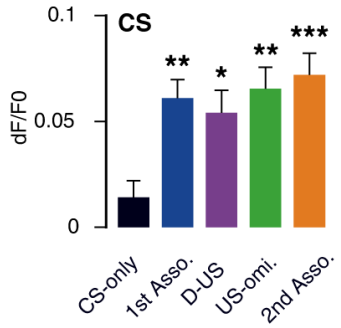
c) Again, to support a 'hierarchical cascade', convincing temporal relationships are needed but not shown in the current data analyses. For example, line27-30 pg 7 mentions that in RSC PLV preceded the rising time point of calcium activities in RSC engrams. But temporal relationship between ca signals and ephys are non-trivial. Moreover, no temporal relationship with VTADA, LEC and other neocortical regions are reported yet seem critical.

We acknowledge the challenge of comparing the time scales of electrophysiological signals and calcium signals, which exhibit different kinetics. Our data indicate that the Engram cell population shows a stronger correlation with PLV signals (**Fig. 7h**). By comparing the latency in the rising times of PLV signals and Engram calcium activity, we found that Engram calcium activity lagged behind PLV by an average of 210 ms during learning (**Fig. 7i**). Given that the averaged delay of GCaMP6f sensor relative to spikes is 50-70 ms (Chen, TW., Wardill, T., Sun, Y. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature.2013), we remain confident in our conclusion that PLV precedes Engram activity on day 3.

In addition to the correlation study, we included a loss-of-function experiment as suggested by Reviewer1#. We delivered hM4D in bilateral LEC and GCaMP6f in RSC of Rbp4-Cre mice (**Extended Data Fig. 9**). CNO was intraperitoneally injected into the animals on day 8 to inhibit LEC activity. Consistent with our model (**Fig. 7j**), our findings indicate that inhibition of LEC did not influence the activity of Engram cells nor impair the behavioral performance of the mice. Therefore, we infer that once the memory Engram network is formed under the modulation of intercortical PLV signals, the reactivation of the Engrams becomes the primary factor in memory retrieval, while the importance of LEC-mediated intercortical synchrony correspondingly decreases. Given the unique projections of LEC[5] to various cortical regions, we hypothesize that mechanisms similar to those observed in the RSC may also exist in other areas.

d) The relationship between LEC and RSC seems somewhat unconvincing. Specifically, Figure 2o-p: the response to CS is weak. In fact it looks more like a reward signal (US) response or expected US? The comparison between 1st Assoc and D-US in Figure 2s and 2v would support this view? Does this not undermine the idea that LEC is carrying a RPE signal to RSC. If recorded across days, is there any evidence for a shift from US to CS across days? 2q provides some evidence to suggest less response to US over time but there isn't evidence for increase in response to CS?

We are sorry for neglecting the analysis of calcium signals in TD-boutons during the CS phase. Compared to the CS-only phase, calcium signals in the TD-bouton group showed significant enhancement during the association training CS period (**Fig. 4m now**). When we intersected the TD-bouton group with the CS-only response group, only 13.4% of TD-boutons exhibited responses during the CS-only phase. This finding indicates that the majority of TD-boutons only developed responsiveness to the CS stimulus after associative learning. We added a description in the main text (**Page 5 Lines 29-33**).



3. Human ECoG data shown in Figure 5. While it is admirable to see cross-species comparisons, I wonder if this data really benefits the rest of the paper. The task is not comparable. Data is then shown for parietal cortex but not frontal and temporal cortices. The reported analyses seems indistinguishable from a surprise or novelty signal as more nuanced RPE characteristics are not considered. The reported effect seems weak and the exact p value is not reported (Figure 5d). It remains unclear how contacts were selected and whether corrections for multiple comparisons were applied.

As we noted in the main text (**Page 8, Lines 10-15**), given the broader definition of prediction error (discrepancies between expectations and perceptions), we believe our task remains informative for interpreting the prediction error signal processing in the human brain.

Our previous analysis yielded relatively weak conclusions regarding the enhanced synchronization within the parietal lobe only, as it was limited to a small subset of channels used in PLV calculation (9 out of 256 for each patient). In the present study, we expanded this analysis by incorporating calculations involving 30 channels selected for each participant. Additionally, one female patient (HS85) whose data did not include parietal lobe ECoG recordings has now been added to the analysis. All patients' electrode placement and the number of pairs used for PLV calculation have been included in the **Extended Table 3 and 4**. We found a broader phenomenon of elevated intercortical synchrony between and within three brain lobes during the 1st block of pseudo-phrases (**Fig. 8c-e**), thereby supporting our conclusion that a broad intercortical synchronized activity characterizes the prediction error signal. The corresponding statistical methods and values are listed in the **Extended Table 1**.

Figure specific queries:

1. Figure 3m onwards: need to make clear in the figure legend which viruses are being expressed and when laser is applied.

We have included this essential information within the corresponding figure legend (**Fig.**

6a now). Thank you for your valuable comments.

2. Figure 4b: lower cranial window image needs AP, ML axes marked. Hard to tell which part of RSC this is. Same for Figure 4c.

We agree with the reviewer's recommendation to incorporate AP, ML axes into the respective figures. Please note that this image has been moved to **Extended Fig. 7a**.

3. For Figure 4c please show contour map (derived from ca signal average across time) of all imaged cells.

We have included representative calcium transient traces for this panel in **Fig. 7c**.

4. For analyses f-I, is there circularity in the definition of engram when comparing to task relevant performance?

In defining Engrams in the RSC region, although we used an experimental method that did not allow simultaneous manipulation while observing, based on Richard Semon's hypothesis on the temporal pattern of Engrams, we identified neurons that are active during both memory encoding and memory recall, as a potential Engrams ensemble to be analyzed subsequently with PLV signals. We found that these cells were more strongly associated with RPE-information-related PLV signals than other neurons, and in turn, this does not imply that cells more strongly associated with PLV are necessarily memory traces. We believe that the logic of these analyses develops linearly.

5. Figure 3d: what about the mCherry control

We included a representative trial-averaged intercortical γ-synchrony spectrogram for the mCherry control experiment in the **Extended Data Fig. 6a**.

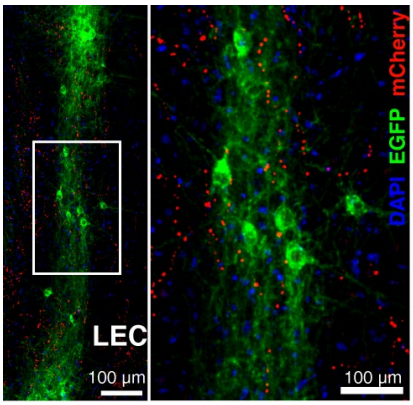
6. Figure 3e: which brain regions are being plotted here?

We apologize for omitting the LFPs recording site information from the figures and figure legends. We recorded electrical signals from the **RSC, M2, and AuD** in related experiments, paired them to calculate PLV values, and finally averaged and compared the PLV values across the three brain region pairs. We have enhanced the description of the recording sites in the schematic and figure legends. Thank you for your valuable suggestion.

7. Figure 3h: at this resolution it isn't possible to tell if axonal boutons deriving from VTA are apposing the cell bodies labelled with GFP in LEC.

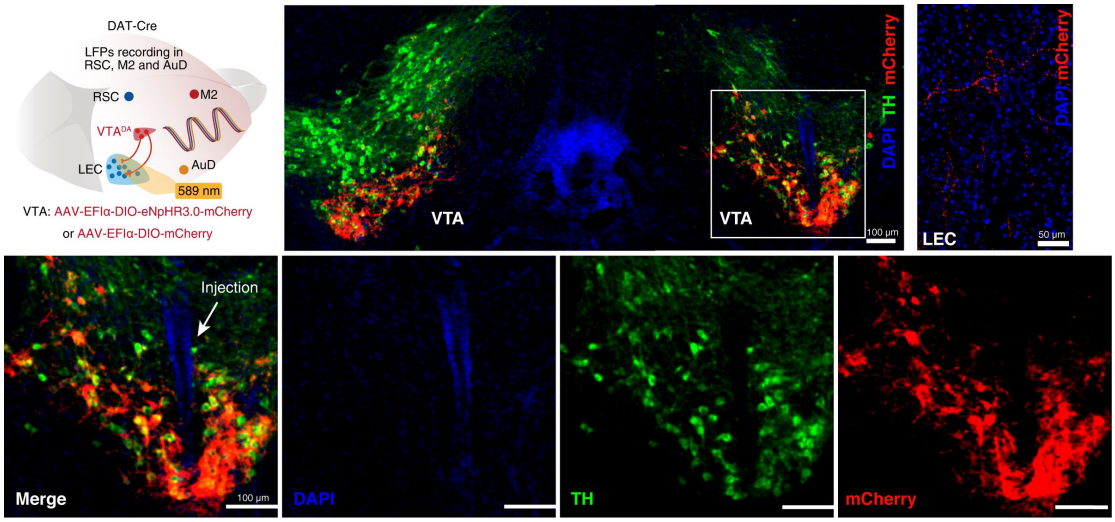
We apologize for not including a zoomed-in confocal image. We have replaced the images with higher-quality ones that clearly demonstrate virus expression in the LEC

region **(Fig. 5h now)**.



8. Figure 3b: At this resolution it is not possible to see overlap between mCherry and TH. Little explanation in main text or figure that this virus is silencing neural activity and that by mCheery the authors mean an mCherry control that doesn't contain the halorhodopsin?

As Reviewer 1# also pointed out, we apologize again for not presenting a zoomed-in confocal image. We replaced the images with higher-quality versions that clearly demonstrate virus expression in the VTA (**Fig. 5b now**). In this experiment, we injected the Cre-dependent optogenetically inhibitory virus AAV-DIO-NpHR3.0-mCherry into the VTA region, and the optic fiber was implanted above the LEC. By stimulating the VTA^{DA} fiber at the LEC with a 589 nm laser, the VTA-LEC functional connectivity was severed. AAV-DIO-mCherry viruses were used in the control group, which do not contain halorhodopsins. A better description of this control group has been included in the main text (**Pages 5 Lines 36-42** and **Pages 6 Lines 1-3**) and the figure legend.



9. Figure 2C-D: which brain regions do these recordings come from?

As in Comment 6 above, we apologize for omitting the LFPs recording site information again. We recorded electrical signals from the **RSC, M2, and AuD** in related experiments, paired them to calculate PLV values, and finally averaged and compared the PLV values across the three brain region pairs. We have enhanced the description of the recording sites in the schematic and figure legends.

10. Figure 2E: what is the p value? What does a negative lag mean given difference in temporal resolution of calcium data and LFP?

Fig. 2e (**Fig. 3e now**) quantifies the discrepancy between the time lag of the VTA^{DA} calcium signal and the increase in intercortical PLV, with the baseline set to zero. $P < 0.0001$. All statistical results from the manuscript have been documented in **Extended Table 1**. A description of this p-value has been included in the corresponding figure legend.

In our original calculation method, we determined the temporal discrepancy between the rising time points of the VTA calcium signal and those of the PLV. Negative values denote that the VTA^{DA} calcium signal precedes the enhancement of cortical PLV, whereas positive values indicate that the cortical PLV enhancement precedes the VTA calcium signal. We note that this negative value could be misleading. As both reviewers pointed out, we revised the methodology for calculating delay to **Lag = RisePLV - RiseVTA**. This modification adjusts the calculated time lag to a positive value. We have also changed the direction of the subtraction calculation in **Fig. 7i**.

11. In Figure 3h it isn't possible to see whether the two viruses are expressed in same location and I couldn't see data for a two laser control using AAV-EF1a-DIO-EGFP and eNpHR3.0 mCherry.

We apologize for not presenting the complete viral injection images. We have replaced the images with higher-quality ones that clearly demonstrate virus expression in the LEC region (**Fig. 5h now**). The trial-averaged intercortical PLV spectrogram for the control experiment is shown in **Extended Data Fig. 6b**. Following Reviewer 1's suggestion, we have changed the data from Day 6 to Day 1 for presentation and analysis (**Fig. 5j-k**).

Reviewer #3 (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.

We sincerely thank you for taking the time to review our manuscript.

Dear Editors and Reviewers,

We would like to express our sincere gratitude for your hard work. Your timely and detailed feedback has greatly improved the quality of our manuscript.