Peer Review File

Manuscript Title:

A metabolic function of the hippocampal sharp wave-ripple

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors demonstrate in rats that clusters of hippocampal sharp wave-ripples (SPWs) are followed within about 10 min by a decrease in tissue glucose levels. Moreover, optogenetic manipulation demonstrates a causal role of SPW-Rs for decreasing glucose levels. The results support the idea of a dual function of SPW-Rs in memory processing and glucose regulation.

The finding is surprising and clearly of interest to the broad readership. The link of SPW-Rs to hippocampal memory processing is well described, and evidence for a parallel contribution to glucose homeostasis deepens our understanding of how the organism adapts to environmental challenges coordinating brain and metabolism. Identifying a potential mechanism directly coupling cognitive processing with metabolic regulation, this study presents truly novel observations that have the potential to substantially advance the field. Nevertheless, I would encourage the authors to more deeply characterize the proposed regulatory metabolic "loop" SPW-Rs contribute to. It needs to be convincingly excluded, for example, that the decrease in glucose following SPW-Rs is simply a globalized (efferent) response to increased energy demands associated with the very high spiking activity accompanying clusters of SPW-Rs.

My comments are specified in the following:

• The experiments focus on effects of SPW-Rs on glucose levels but neglect feedback actions. In fact, the authors mention the possibility of a negative feedback loops acting on the functional SPW-R-glucose axis but have not explored this possibility in their experiments. E.g., the use of glucose (clamp) challenges would be extremely helpful for the characterization of such proposed negative loops. At a first step, glucose "events" (transient increases/decreases) could be identified in the signal and used to analyze time-event-histograms for SPW-Rs. Feedback actions might be detectable only in baseline/resting conditions but not with metabolic challenges which would be also important information.
• Beyond the identification of a metabolic role of SPW-Rs, the studies also fall short of establishing a plausible mechanistic link between SPW-R activity and the regulation of peripheral glucose fluxes. Likely contributions of hypothalamic nuclei to whole-body glucose regulation are mentioned (line 250) but not addressed in the experiments. The same applies to sympathetic and parasympathetic efferences and HPA axis activity (although according to the "author contributions" section it seems that experiments on vagus nerve stimulation were performed). Information about insulin concentrations would be essential for a complete characterization of peripheral glucose homeostasis.
• In addition to the gain-of-function optogenetic experiment, loss-of-function studies would be highly desirable to buttress the assumption of a critical role of SPW-Rs in maintaining (long-term) glucose homeostasis.

• As to the association of SPW-Rs with NREM sleep, the authors refer to experiments that have shown a disruption of insulin sensitivity after three nights of SWS suppression in healthy individuals (ref. 51). This study is still one of the few reliably demonstrating a specific contribution of NREM sleep – rather than sleep per se – to metabolic control. In contrast, there is a plethora of findings that indicate adverse glucoregulatory effects of shortened/desynchronized sleep (while SWS duration is largely unchanged), suggesting that other sleep stages are more important.

Minor points:

• Line 56 SPW-R's are linked to consummatory actions. How does this fit with the assumed role of SPW-Rs as indicator of episodic memory processing and planning (ref 11)?
• Line 159 "Short duration, i.e., likely more synchronous, SPW-Rs" I would link "more synchronous SPW-Rs" to amplitude rather than duration?
• Line 200 (also Methods). A total injection volume of 300 µl of virus seems too much. Please check.
• Line 210 "In seven of …. The effect on glucose concentration was larger at high stimulation rates (Figure 6E and F, average $R = -0.34$; $P < 0.001$)". What does the statistics refer to? Fig. 6D shows a distinctly lower peak negative cross correlation around -0.09. Please clarify.
• Line 224, "magnitude" - the correlation is not very high.
• Section NREM sleep and glucose homeostasis – referencing could be updated.
• The translational relevance of the findings could benefit from the use of rat models with pharmacologically or diet-induced reductions in insulin sensitivity/glucose tolerance.
• Optogenetics: Histology of local virus expression and electrode positioning should be included.
• Ripple induction experiments: viral injections were targeted to CA1 (5 rats) and to CA3 (3 rats). Did stimulation protocols and ripple quality differ depending on which structure was targeted.
• Fig. 6 indicates that ripples were induced at a rate of 0-40 ripples per min for two days. Please clarify whether only "induced ripples" were analysed and how "natural occurring ripples" during this two day period were excluded from the analysis.
• Why are data shown in Figure 6E &F at +10-30 min instead of +10 min as in Fig. 4F &G?
• Which brain states were differentiated (wake vs NREM, REM, preREM?), and how (criteria)?
• P.10: It is not clear how the models were trained exactly. The authors describe a 100-fold cross validation procedure, as well as a split of the data set into three parts. Please clarify.
• Typos: P.5 Figure 1A Caption: 1.25 kHz instead of "1250 kHz"; Extended Data Figure 7: Panel B should be "zeitgeber"

Referee #2 (Remarks to the Author):

Tingley and colleagues reveal that hippocampal sharp wave-ripples are reliably associated with a future decrease in peripheral glucose levels. The findings are novel and important, and may provide a neurophysiological mechanism for associations between sleep disruption and diabetes and obesity. Below I describe areas that could be improved upon. My primary concerns (#6 and #8 comments below) relate to the lack of mechanistic insight with regards to the lateral septum as a downstream mediator, and the lack of insight into the actual relevance of the data in the context of diabetes and obesity.

1. Generally speaking, the authors should make it abundantly clear throughout the manuscript (and early on) that they are recording from dorsal CA1. The word "dorsal" was not even mentioned until line 110, and CA1 was not mentioned until Figure 1 legend. Related, I did not see coordinates for the electrode placements (or AAV injections) in the Methods section.

2. The figure captions are quite passive/descriptive and do not describe to the reader the take-home key finding represented in the figure (with the possible exceptions of Figures 5 and 6).

3. Figure 3: Up until this point of the manuscript it appeared as if the chronic ephys recordings were occurring in ad lib-fed animals in the home cage/apparatus, as described in the methods. However, in Fig. 3 the meals are described as "one 3-gram pellet of standard chow". This needs to be clarified. Were the animals food restricted and given one, or multiple 3-gram meals? Related, when were these meals (or meal?) given? Does the circadian fluctuation in panel C take into account the meals (or meal), or was this recording taken over a period without food access? Overall the authors do a poor job of communicating the analyses as they relate to meals / food intake, which is not a trivial component of the manuscript given that the main findings relate to regulation glucose levels in the periphery.

4. Extended Data Fig. 5 is critical and should be included in the main part of the text. However, the legend poorly describes the figure and it is not clear what is going on. First of all, the acronyms CGM1, DT12, and flex1 should be included in the legend. Second, it is again unclear how the authors are analyzing ripple rate and delta current specifically for the meal. How long are the meals? Important question: to what extent is the negative correlation between SPW-R and future glucose concentrations stronger or weaker during various stages of activity (e.g., sleep vs. eating vs. awake but not eating vs. immediately postprandial, etc.)? This was not clearly elaborated on. The authors conclude that short duration SPW-Rs are associated with the largest decrease in future glucose concentrations, but from the way the data are presented and described, it is unclear to what extent the behavior of the animal (e.g., eating) influences this relationship. Part of my confusion relates to comment #2 above, as in many cases the figure legends do not do a good job of communicating the main result(s) of the figure.

5. The conditions for the optogenetics experiment in Fig. 6 are not clear, from either the Results or the Methods. Did the animals have access to food, or was food withheld? Also, the authors should include photomicrographs showing AAV injection sites for the ChR2.

6. The lateral septum data were interesting, but then nothing was followed-up on. Is LS activity also related to interstitial glucose concentrations? Would LS lesions (or inactivation) block the relationship between CA1d SPW-R and glucose?

7. Related to #6, it was not clear exactly why the authors chose dorsal CA1 and the LS as targets of interest in the first place (vs. other hippocampal regions, and/or other downstream targets). They mention that the LS is "the major relay nucleus between hippocampal-subicular-entorhinal system and the hypothalamus". However, the ventral CA1 has direction projections to BOTH the LS and the hypothalamus (e.g., lateral region).

8. The authors hypothesize that their results may relate to the connection between sleep quality and T2 diabetes and/or obesity. This is clearly a testable hypothesis that would greatly enhance the paper: is the hippocampal SPW-R – glucose relationship altered in diabetic and/or obese rats.

Referee #3 (Remarks to the Author):

Tingley et al. investigate correlation between sharp wave ripple rate and interstitial glucose concentration in rats with the view of assessing whether hippocampal SWRs may also support a

general metabolic function. The authors find periods characterised by high (>100 rip/5min) SWR rate are associated with low glucose concentration. Further, the authors show this negative relationship between SWR rate and glucose concentration can be artificially reinstated by optogenetically inducing SWRs. Finally, although circadian modulation of SWR rate and glucose concentration is anti-correlated (periods of high glucose concentration during a 24hr light cycles are associated with a lower SWR rate (fig2E, fig3c)) the authors claim the relationship between SWR rate and glucose concentration cannot be merely accounted for by vigilance state (NREM vs awake). These findings are undeniably novel and if true then this would challenge traditional theories of SWR function and could lead to a novel research direction.

However, I remain to be convinced the relationship between SWR rate and glucose levels is not just a result of changes in vigilance/brain state. The paper's conclusions would benefit from a number of further analyses to eliminate this alternative explanation. Moreover, more information about their glucose measurement and state scoring method is required. I outline each of my concerns below.

- It seems the majority (>75%) of SWRs occur during NREM sleep, however could the authors show the relationship between glucose concentration and SWR rate is maintained if only awake SWRs are analysed? If it is, this would provide a more convincing evidence that SWR rate x glucose correlation is not just mediated by brain state (e.g. NREM sleep).
- Could the authors differentiate between different stages of NREM sleep. Rats, similar to humans, go through different stages of NREM sleep (e.g. see Lacroix et al. (2018). High ripple rates may reflect deep NREM sleep specifically (as indeed mentioned by the authors in the discussion). Thus, perhaps the SWR rate x glucose correlation can be explained by depth of NREM. The authors need to address this confound.
- Related to this, the methods section on sleep scoring was very brief. It is not clear how the authors are differentiating between NREM and awake states. Further, NREM vs awake is a very crude distinction and perhaps is why the awake/nrem predictor in their model is not able to capture as much of the variance in glucose levels as SWR rate.
- Although the authors show inducing ripples also leads to a reduction in glucose, this effect is substantially weaker than that found for spontaneous ripples (e.g. if you compare fig4F and 6E). Moreover, inducing ripples at a high rate may affect oscillatory activity in down-stream cortical networks. Could the authors show synthetic ripple clusters do not lead to NREM-like cortical oscillations (e.g. delta waves).
- The description of the glucose concentration measurement method is very brief. Could the authors provide more details here, for example the sampling rate of glucose levels is not given.

Minor comments:
- There are a number of typos in the figures and legend. e.g. fig1c - one line is meant to be blue and the other red, but currently both are red (making it very hard to interpret the figure). There are similar errors in other figure panels in the manuscript.

Author Rebuttals to Initial Comments:

We would like to thank the Reviewers for their constructive critique of our manuscript. Each reviewer acknowledged the novelty and significance of our findings (Rev 1: "*The finding is surprising and clearly of interest to the broad readership.*" Rev 2: "*The findings are novel andimportant, and may provide a neurophysiological mechanism for associations between sleepdisruption and diabetes and obesity*." Rev 3: "*These findings*

are undeniably novel and if truethen this would challenge traditional theories of SWR function and could lead to a novel research direction.”). However, the Reviewers also objected against some of our conclusionsand suggested additional experiments to improve presentation and clarity.

We have spent the past several months performing these experiments. Given the magnitude of the requested tasks, we have recruited a graduate student (Jordan Carpenter) to assist the original authors to accomplish our goals. These included 1) loss of function experiment by pharmacogenetically suppressing the LS, the hypothesized conduit of hippocampal output to subcortical structures, 2) optogenetic stimulation of the neocortex as a specificity control, 3) recording of SPW-Rs in dorsal and ventral hippocampal regions simultaneously, and 4) further analyses at multiple points in the manuscript. In essence, we have more than tripled the numberof observations of the primary phenomenon (N = 30 out of 30), expanded the manuscript in several directions which helped to address the main concerns of the Reviewers, and increased statistical power for our original claims. The overall result, we believe, is a strong manuscript with substantially more support for the role of SPW-Rs in regulating peripheral glucose levels.

Below, we specifically address each Reviewer’s comments/suggestions. Major changes in the manuscript are highlighted.

Referee #1 (Remarks to the Author):

The authors demonstrate in rats that clusters of hippocampal sharp wave-ripples (SPWs) are followed within about 10 min by a decrease in tissue glucose levels. Moreover, optogenetic manipulation demonstrates a causal role of SPW-Rs for decreasing glucose levels. The resultssupport the idea of a dual function of SPW-Rs in memory processing and glucose regulation.

The finding is surprising and clearly of interest to the broad readership. The link of SPW-Rs to hippocampal memory processing is well described, and evidence for a parallel contribution to glucose homeostasis deepens our understanding of how the organism adapts to environmentalchallenges coordinating brain and metabolism. Identifying a potential mechanism directly coupling cognitive processing with metabolic regulation, this study presents truly novel observations that have the potential to substantially advance the field. Nevertheless, I would encourage the authors to more deeply characterize the proposed regulatory metabolic “loop” SPW-Rs contribute to. It needs to be convincingly excluded, for example, that the decrease in glucose following SPW-Rs is simply a globalized (efferent) response to increased energy demands associated with the very high spiking activity accompanying clusters of SPW-Rs.

If we interpret the Reviewer’s concern correctly, the potential critique here is that glucose utilization (thus, decrease) is due to the energy cost of SPW-R-related spiking per se and this cost is reflected indirectly as a peripheral glucose level reduction. We can confidently exclude this explanation. First, the energy cost of spiking during nonREM sleep (a SPW-R rich brain state) is typically less than in the waking brain. Although the brain’s energy budget is generally high (20% of the entire organism in humans), the energy cost differential between brain states, compared to the energy budget of the body, is very small (Attwell and Gibb, 2005). However, weagree that this response is very indirect. To provide a quantitative

answer, we calculated the spiking activity surrounding SPW-Rs in the relevant time windows, using 1-second and 3-minutetime bins (Figure below). The overall spiking activity at the minute temporal scale is actually decreased. While at very short time scales (< 100 milliseconds) the correlation is positive, at all timescales longer than 250 milliseconds we found an inverse correlation. The reason is that even though the SPW-R is the most synchronous network event in the mammalian brain (due toprecise time alignment of spikes of neurons) it occurs *without* increasing firing rates of individualpyramidal cells (Buzsáki et al., 1992). Importantly, each population burst is followed by a longer spike-poor period (English et al., 2014), thus action potential firing is more concentrated but not higher on average during clusters of SPW-Rs as compared to other brain states (i.e. theta; (Csicsvari et al., 1999)).

A related experiment to this issue is the observation that during spike and wave epilepsy (with analogous synchronized discharge of the thalamus followed by longer silence periods) is associated with decreased BOLD in human subjects (Aghakhani et al., 2004).

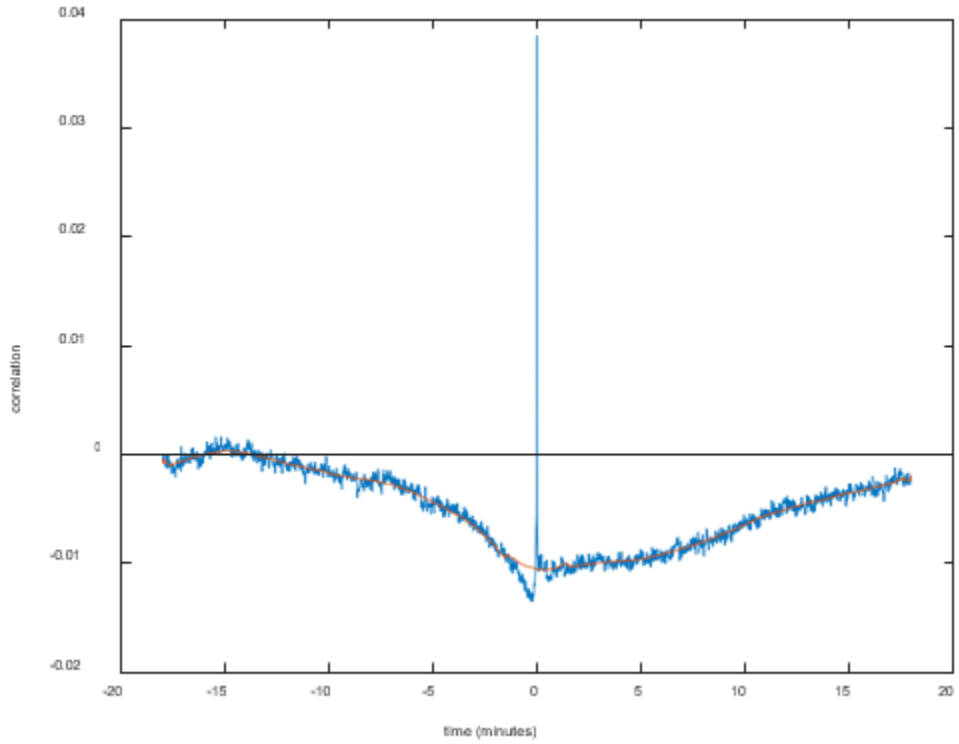


Figure. Correlation between SPW-R (time zero) and spiking activity of dorsal CA1 neurons. Blueline uses 1-second bins; red line uses 3-minute bins. Now incorporated as Extended Data Figure 4E.

Another way to address the Reviewer's concern, we conducted an additional experiment wherewe optogenetically induced ripples in the posterior parietal cortex. The same optogenetic stimulation protocol as used in our CA1 animals, induced ripple-like population bursts in neocortex when virus expression and optogenetic stimulation was applied in the neocortex (Stark et al., 2014). These induced non-hippocampal population bursts (in posterior parietal cortex), presumably involving a similar number of spikes and neurons in each event, were unable to drive decreases in glucose levels in the periphery (Extended Data Figure 12).

Finally, our new pharmacogenetic experiments, blocking spike transmission through the lateralseptum (Figure 5) also suggest that the direct metabolic cost of SPW-Rs is not driving the decrease in peripheral glucose levels, as SPW-R rate was not altered during CNO injection days even though the SPW-R/glucose correlation was decreased to chance levels.

My comments are specified in the following:

• The experiments focus on effects of SPW-Rs on glucose levels but neglect feedback actions. In fact, the authors mention the possibility of a negative feedback loops acting on the functional SPW-R-glucose axis but have not explored this possibility in their experiments. E.g., the use of glucose (clamp) challenges would be extremely helpful for the characterization of such proposed negative loops. At a first step, glucose “events” (transient increases/decreases) couldbe identified in the signal and used to analyze time-event-histograms for SPW-Rs. Feedback actions might be detectable only in baseline/resting conditions but not with metabolic challengeswhich would be also important information.

We wholeheartedly agree with the Reviewer that exploring the entire loop of brain-body regulation will be needed to nail down the full impact of SPW-Rs on peripheral glucose levels (and putative feedback from the body). We did not address the glucose feedback partially because this has been well supported by the available literature (Gold, 2005; Korol and Gold, 1998). Several papers have already shown that the hippocampus is the most ideal brain sensor(it is ‘floating’ in the lateral ventricles) with several-fold higher concentration of many receptors compared to neocortex and subcortical structures (with the exceptions the hypothalamus; (Lein et al., 2007)). Of course, anatomical data do not address regulatory issues and we agree that the suggested glucose clamp would be very useful. Unfortunately, performing such experimentsin freely behaving animals is beyond our current expertise (requiring indwelling catheters and frequent blood sampling in freely behaving rats (Hughey et al., 2010)). As the Reviewer likely sensed it, we have only recently begun branching out to the body.

In an attempt to address the idea of feedback within this putative loop at least partially, we haveincluded the suggested ‘reverse’ correlation analysis (Extended Data Figure 7). In this analysis,we identified large (> 1 standard deviation) peaks/troughs in the glucose signal and examined the average peri-event SPW-R rate surrounding these peaks or troughs. Both event types showa ‘ringing’ with a cycle time of ~60 minutes, suggesting there is at least some mild form of feedback (also note that this matches the leftward positive correlation seen in Figure 2E).

In addition, we have done this analysis also for our optogenetically induced ripples, which demonstrated further that our synthetic ripples were dissociated from possible brain state-dependent glucose level fluctuations (i.e. a lack of ‘ringing’).

• Beyond the identification of a metabolic role of SPW-Rs, the studies also fall short of establishing a plausible mechanistic link between SPW-R activity and the regulation of peripheral glucose fluxes. Likely contributions of hypothalamic nuclei to whole-body glucose regulation are mentioned (line 250) but not addressed in the experiments. The same applies tosympathetic and parasympathetic efferences and HPA axis activity (although according to the “author contributions” section it seems that experiments on vagus nerve stimulation

were performed). Information about insulin concentrations would be essential for a complete characterization of peripheral glucose homeostasis.

Our unexpected discovery is that hippocampal SPW-Rs are capable of modulating peripheral glucose regulation and we focused on buttressing the evidence in support of this major claim. We have further extended this to show that *the first step* in a candidate circuit involves the lateral septum (Figure 5) which heavily projects to several hypothalamic and brainstem regions.The clarification of the exact effector mechanisms within and beyond the hypothalamus will have to wait for careful future experiments. It should be noted, however, that HPA-axis and sympathetic/parasympathetic signaling are often *highly* redundant [see e.g., figure 2 of Shimazu1987(Shimazu, 1987) or an updated version (Güemes and Georgiou, 2018)].

In a set of early pilot acute experiments, we attempted to record from and stimulate the vagalnerve but we were unable to convince ourselves that this could be done reliably even in an anesthetized preparation (current vagal nerve recording studies often use an anesthetized preparation to reduce muscle artifact(Zanos et al., 2018)). Even after establishing these complex new methods in our laboratory, we would have faced the issue of specificity of vagalstimulation, and thus chose to pursue alternative experiments.

We also agree that monitoring insulin concentrations would be another way to better characterize glucose homeostasis. Unfortunately, no fast, chronic monitoring methods currentlyexist (that we are aware of), comparable to the glucose sensor we used. Electrochemical aptamer-based biosensors do show some promise, and the first author (Tingley) has discusseddeveloping such a tool with several biochemistry laboratories for future use.

Prior to identifying the continuous glucose sensor used in our experiments, we attempted to sample blood from the femoral vein chronically. Unfortunately, after several months of work, wewere limited to intermittent sampling of blood (at best ~8 timepoints per day, with a 7-day recovery period; NIH Guidelines for Survival Bleeding of Mice and Rats) in behaving animals before the blood volumes required for testing exceeded the maximum allowable volume which can be removed during a survival experiment. Thus, we were unable to obtain good enough temporal resolution and the process of sampling inevitably interfered with physiological sleep states.

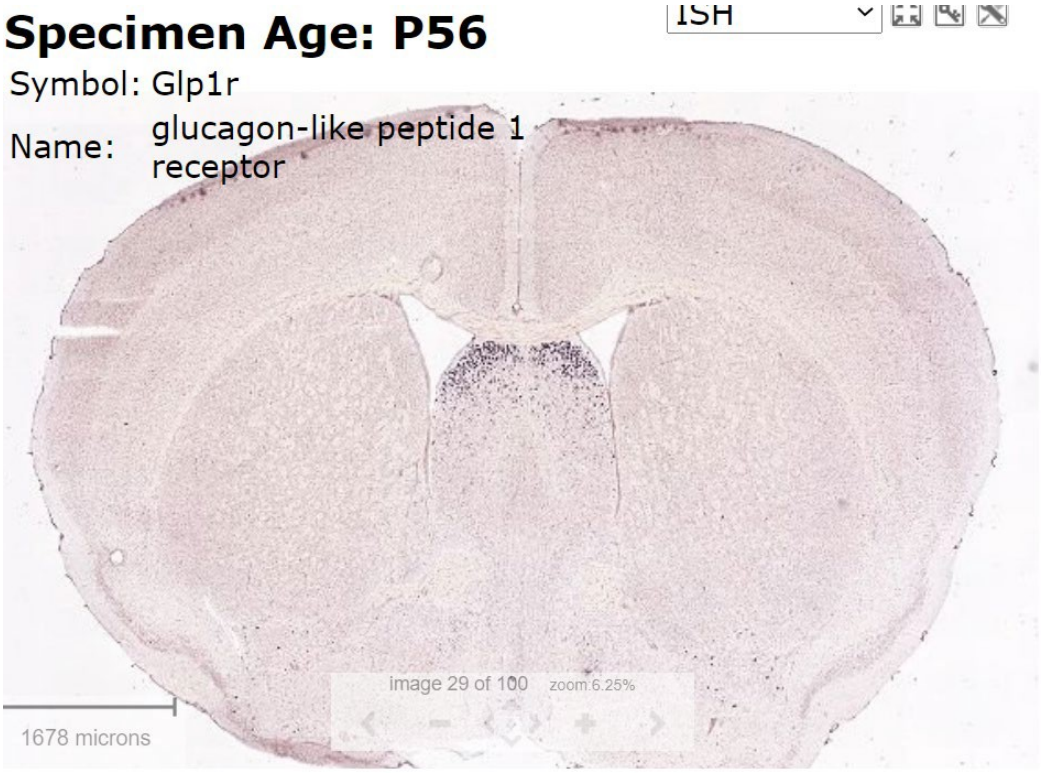
We wish we could replace this apologetic response to the Reviewer with targeted experiments, but we are not able to provide answers in a reasonable time epoch. The lead author has chosento continue research in brain-body issues and will do a post-doc in a new lab, where he will explore the development of such needed methods.

• In addition to the gain-of-function optogenetic experiment, loss-of-function studies would behighly desirable to buttress the assumption of a critical role of SPW-Rs in maintaining (long- term) glucose homeostasis.

Here, we are in a better position. Prompted largely by the Reviewer's comment, we performed the needed experiments. To test our hypothesis that one possible conduit of hippocampal SPW-R effect is through the lateral septum, we used a pharmacogenetic approach to silence the lateral septum. We found that during pharmacogenetic blockade of the lateral septum, the correlation between SPW-Rs and glucose fluctuation decreased to chance levels. Perhaps equally importantly, this loss-of-function perturbation experiment

provides further support for therole of the hippocampus in controlling peripheral glucose fluctuation.

Our choice of the lateral septum as an intermediate structure between hippocampus and hypothalamus was prompted by studies that showed that the lateral septum (specifically the dorsal region) expresses an extremely high level of GLP1 receptors (in fact, the highest of all brain regions), which alter dLS excitability (see figure below taken from Allen Institute ISH data, and (Harasta et al., 2015; Terrill et al., 2016, 2019)). Both glucagon and GLP1 can bind this receptor (glucagon has a lower affinity (Runge et al., 2003)), and it has also been shown that glucagon-like peptide (GLP1) and glucagon both cross the blood-brain barrier (Banks and Kastin, 1985; Kastin et al., 2002). The emerging view from these studies is that dLS excitability may be modulated by peripheral fluctuations in GLP1 and glucagon, thus changing how dLS responds to hippocampal inputs. A potential prediction from this observation is that HPC-LS coupling strength during SPW-Rs (Tingley and Buzsáki, 2020) may be hormone-level dependent, and in turn alter the ability of SPW-Rs to reach peripheral metabolic effectors, whichmay itself be under hormonal regulation. Thus, in addition to the hippocampus, the lateral septum is both an effector and a sensor in this potential loop.



• As to the association of SPW-Rs with NREM sleep, the authors refer to experiments that haveshown a disruption of insulin sensitivity after three nights of SWS suppression in healthy individuals (ref. 51). This study is still one of the few reliably demonstrating a specific contribution of NREM sleep – rather than sleep per se – to metabolic control. In contrast, there is a plethora of findings that indicate adverse glucoregulatory effects of shortened/desynchronized sleep (while SWS duration is largely unchanged), suggesting that other sleep stages are more important.

We deleted the sentence related to ref. 51. We agree with the Reviewer that it is possible that both REM and nonREM contribute to glucose regulation one way or another. However, given that nonREM/REM ratio is 4:1, it places nonREM into a favorable position. No SPW-Rs are present during REM sleep so our findings support the importance of SPW-R-rich nonREM. REMsleep episodes in rodents last typically <1 min, which is considerably shorter than the temporal resolution of our glucose monitor. In humans, REM sleep is accompanied by increased cerebral glucose utilization and cerebral blood flow (Abrams et al., 1988; Maquet, 2000) and adecreased concentration of insulin and glucagon (Braun et al., 1997).

Short of our ability to examine sleep stage-specific glucose levels, we used a continuous variable ("power spectrum slope"; (Donoghue et al., 2020) as a candidate predictor of glucose fluctuations. We show that this continuous brain-state variable was one of the least informativepredictors of peripheral glucose level fluctuations (Figure 3).

Minor points:

• Line 56 SPW-R's are linked to consummatory actions. How does this fit with the assumed roleof SPW-Rs as indicator of episodic memory processing and planning (ref 11)?

Memory consolidation is a protracted offline process, taking place after the learned episodes. Itshypothesized role in planning is similar, as a 'non-conscious' retrieval process whereby internally generated neuronal assemblies are matched to the current situation (Buzsáki, 2015). We have expanded the final paragraph of the manuscript to further describe how we view the role of SPW-Rs in linking the consolidation of experience and regulation of metabolic processes.

• Line 159 "Short duration, i.e., likely more synchronous, SPW-Rs" I would link "moresynchronous SPW-Rs" to amplitude rather than duration?

We apologize for the phrasing here and have simplified the text. There is a multi-variate correlation at play here. Often, the duration of a SPW-R is related to the amplitude, instantaneous frequency, and inter-ripple-interval. We were attempting to allude to the fact thateach of these SPW-R 'features' is correlated with the others.

• Line 200 (also Methods). A total injection volume of 300 µl of virus seems too much. Pleasecheck.

Thank you for catching this. The correct volume was 300 nL. We have corrected this in the revised version. We used three DV positions, 100nL each.

• Line 210 "In seven of …. The effect on glucose concentration was larger at high stimulationrates (Figure 6E and F, average R = -0.34; P < 0.001)". What does the statistics refer to? Fig.6D shows a distinctly lower peak negative cross correlation around -0.09. Please clarify.

These statistics originally referred to binned data (across ripple rate), effectively smoothing out much of the variability. We agree with the Reviewer that this was confusing and have changed these histograms (now Figures 2F and 4E) to reflect the correlation from the non-binned raw data (i.e., the peaks in the average CCG for 2E and 4D are now reflected as the means of eachhistogram). We have also changed Figure 4D (and 2E) to reflect the median

rather than mean cross-correlogram changing the peak correlation from R = -0.09 to -0.11.

• Line 224, “magnitude” - the correlation is not very high.

Strictly speaking every number of a correlation is a ‘magnitude’. The average correlation was –0.21. However, we agree with the Reviewer that the term ‘magnitude’ has the connation of ‘big’.Therefore, we deleted this word from that sentence. We would also like to note that in our new experiments, we tested an improved continuous glucose monitoring device (Dexcom G6) in tworats, which has been shown to be more accurate. In these animals the correlation was higher (R = -0.3) suggesting the possibility that our reported correlation of R = -0.2 may be an underestimate of the physiological response due to measurement noise. In addition to magnitude, another important characterization of correlation is consistency and replicability. Wehave seen the SPW-R-related glucose dip in 30 out of 30 tested rats.

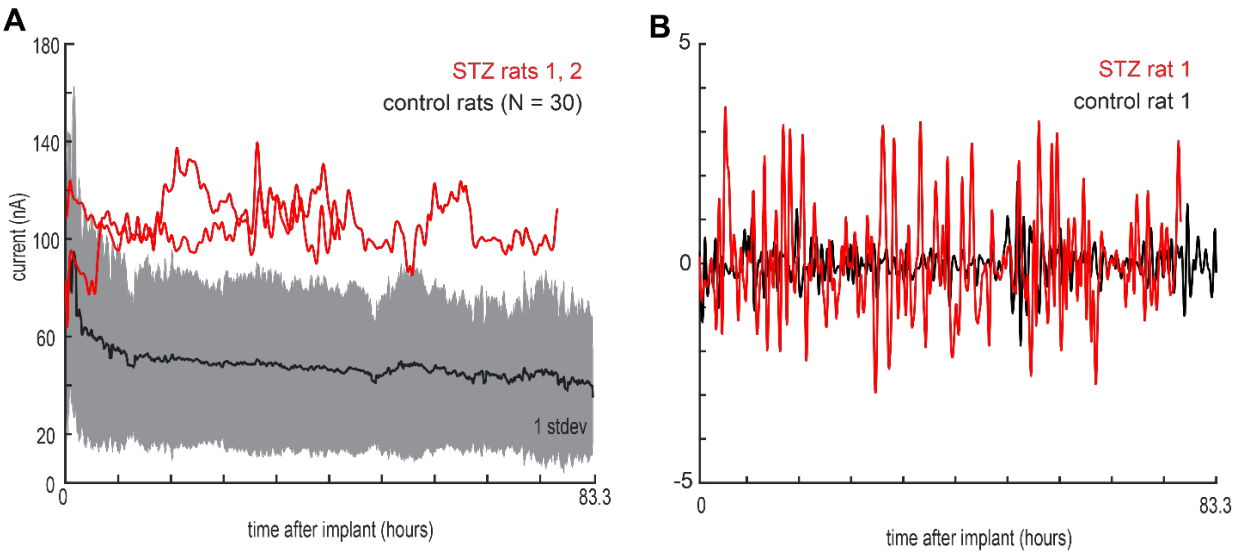
• Section NREM sleep and glucose homeostasis – referencing could be updated.

We have initially listed a dozen or so references (Buxton et al., 2010; Cappuccio et al., 2010; Donga et al., 2010; Gangwisch et al., 2007; Leproult and Van Cauter, 2010; Nedeltcheva et al.,2009; Schmid et al., 2009, 2011, 2015; Spiegel et al., 1999) but had to choose from among these equally good studies to keep the references within the limit set by Nature instructions. Weadded Spiegel et al., 1999 to the revised manuscript because it is among the earliest ones and with the highest impact on the field. If the Reviewer has an alternative suggestion(s), we will be happy to include those.

• The translational relevance of the findings could benefit from the use of rat models withpharmacologically or diet-induced reductions in insulin sensitivity/glucose tolerance.

We agree with the Reviewer that such extension of the work would be an important step. As noted later in our response letter, body weight did not significantly affect the correlation we report. However, expanding this to animals with altered diets, or disease models, will be important future experiments. We believe that there are several qualified and interested groupswho can do such work with more precision.

Even though this was a minor point of the Reviewer, we attempted to follow their advice. In 3 rats, we pharmacologically destroyed insulin-producing cells in the pancreas by streptozotocin injection. This is a standard type I diabetes rodent model (Wei et al., 2003). Our naïve working hypothesis was that after elimination of the beta cells of the pancreas, that the peripheral glucose rhythm which is so prominent at the 60-minute scale (Fig. 1B; Extended Data Figure 2D-E) would no longer be present for SPW-Rs to phase reset (Fig. 2H). The procedure workedeffectively as shown by the two to three-fold increase of absolute glucose levels (glucose levels >250mg/dL). However, to our surprise and disappointment, the 60-minute fluctuation, did not disappear but, instead, was enhanced substantially (panel B below; avg. standard deviations were 1.43 nA vs 0.39 nA). This finding tells us that multiple peripheral factors (potentially including glucagon fluctuations and/or liver mechanisms) can support the glucose rhythms whose peripheral regulation needs to be better understood in future experiments. There may beother ways to eliminate the glucose fluctuation (such as glucose clamp or denervation of peripheral organs) but such methods are beyond our current abilities.



(A) Current readings from the iPro2 device relative to the amount of time after placement. Black line is the average current readings from 30 intact rats, showing the slow decrease which corresponds to enzyme (glucose oxidase) breakdown on the electrode. Red lines are two STZ animals, showing glucose levels ~2.5-fold higher that control animals. **(B)** The variability in glucose levels was substantially larger in STZanimals.

• Optogenetics: Histology of local virus expression and electrode positioning should be included.

As suggested, we have included histology for recordings, optogenetic, and pharmacogenetic experiments.

• Ripple induction experiments: viral injections were targeted to CA1 (5 rats) and to CA3 (3 rats).Did stimulation protocols and ripple quality differ depending on which structure was targeted.

The stimulation protocol was the same for all animals and both CA1 and CA3 are capable of producing ripple-band population bursts when stimulated. We did not find significant differencesbetween these groups and this is why we grouped them into a single cohort. The initial hypothesis was that driving CA3 may lead to a larger effect as it 1) projects to CA1 and drives SPW-Rs there, which di-synaptically reach LS, 2) more densely projects to LS directly, and 3) projects to the other hemisphere thus allowing for a bilateral population burst. Given that CA3 and CA1 regions send convergent projections to the dorsal lateral septum (Figure 5A), stimulating either region may act on the same downstream pathway to produce a similar population burst in LS, leading to similar effect sizes.

• Fig. 6 indicates that ripples were induced at a rate of 0-40 ripples per min for two days. Pleaseclarify whether only “induced ripples” were analysed and how “natural occurring ripples” during this two day period were excluded from the analysis.

Each induced ripple (i.e. current pulse) had a timestamp, which was recorded separately from the electrophysiology (as an additional analog signal). We used these timestamps for testing thecorrelation between artificially-induced ripples and glucose concentration. Our goal was to

match the overall statistical distribution of the induced ripples to the naturally occurring ones (detected in different sessions) so that we could dissociate the timing of the artificially-induced and naturally occurring events. The exact stimulation protocol is now described in detail in the Methods section.

• Why are data shown in Figure 6E &F at +10-30 min instead of +10 min as in Fig. 4F &G?

We apologize for this discrepancy and have made the appropriate changes. We had originally conducted this analysis in two ways, the first was taking a single 5-minute bin at the offset with the largest negative correlation (+10 minutes) and the second was to take a time window (10-30minutes) in an attempt to capture all timepoints where the correlations were significant. These two approaches resulted in nearly identical plots however we forgot to standardize across different datasets (original recordings vs optogenetic experiments). We have updated this such that both figures have been recalculated using the first (single bin) approach. With the addition of 22 animals in Figure 2G (previously Figure 4G), the average effect of ~40 ripples has increased from -0.4nA change to -0.6nA.

• Which brain states were differentiated (wake vs NREM, REM, preREM?), and how (criteria)?

We used four different metrics to quantify 'brain state'. One of these metrics was simply a Wake/nonREM differentiation (0 or 1) determined using a semi-supervised sleep scoring algorithm (Watson et al., 2016). As noted above, REM sleep in the rodent is typically ~1 min long, which is shorter than the temporal resolution of the glucose monitor, reducing the potentialfor comparing REM with nonREM. As an alternative approach, we also used other metrics to quantify brain state, including theta/delta ratio, power spectrum slope, and theta residual when subtracting the power spectrum linear fit (i.e. theta power). All three of these metrics are continuous variables. We now describe these measures better in the Supplementary Methods.

• P.10: It is not clear how the models were trained exactly. The authors describe a 100-foldcross validation procedure, as well as a split of the data set into three parts. Please clarify.

The dataset was split in two parts. Each model was trained on 2/3^{rds} (67%) of the dataset, and tested on the remaining 1/3rd (33%). The selection of 2/3^{rds} and 1/3rd was random (using Matlabrandperm.m) and 100 different random selections were conducted for testing and training.

• Typos: P.5 Figure 1A Caption: 1.25 kHz instead of "1250 kHz"; Extended Data Figure 7: PanelB should be "zeitgeber"

We thank the Reviewer for alerting us to these typos. We corrected them and a few others thatwe identified during the revision process.

Referee #2 (Remarks to the Author):

Tingley and colleagues reveal that hippocampal sharp wave-ripples are reliably associated with a future decrease in peripheral glucose levels. The findings are novel and important, and may provide a neurophysiological mechanism for associations between sleep disruption and

diabetes and obesity. Below I describe areas that could be improved upon. My primary concerns (#6 and #8 comments below) relate to the lack of mechanistic insight with regards to the lateral septum as a downstream mediator, and the lack of insight into the actual relevance ofthe data in the context of diabetes and obesity.

1. Generally speaking, the authors should make it abundantly clear throughout the manuscript(and early on) that they are recording from dorsal CA1. The word “dorsal” was not even mentioned until line 110, and CA1 was not mentioned until Figure 1 legend. Related, I did notsee coordinates for the electrode placements (or AAV injections) in the Methods section.

We have added specification of dorsal CA1 throughout the manuscript and have also performedrecordings from both the dorsal segment and the ventral tip of the hippocampus (Extended DataFigure 13). We spell this out early in the manuscript, as suggested by the Reviewer. The exact coordinates for each animal are available as a table in the Supplementary Information section (typically -4AP, 3ML for dorsal CA1).

2. The figure captions are quite passive/descriptive and do not describe to the reader the take-home key finding represented in the figure (with the possible exceptions of Figures 5 and 6).

We tried to follow Nature’s recommended format. Yet, prompted by the Reviewer’s comment, we spell out the key relevance of each figure in the revised version.

3. Figure 3: Up until this point of the manuscript it appeared as if the chronic ephys recordings were occurring in ad lib-fed animals in the home cage/apparatus, as described in the methods. However, in Fig. 3 the meals are described as “one 3-gram pellet of standard chow”. This needsto be clarified. Were the animals food restricted and given one, or multiple 3-gram meals? Related, when were these meals (or meal?) given? Does the circadian fluctuation in panel C take into account the meals (or meal), or was this recording taken over a period without food access? Overall the authors do a poor job of communicating the analyses as they relate to meals / food intake, which is not a trivial component of the manuscript given that the main findings relate to regulation glucose levels in the periphery.

In the revised version we provide all necessary details. As suggested by the Reviewer, the fed/fasting experiments are now part of a main figure. The fed/fasting experiments (now Figure 2I-K) were a separate set of recordings that are not included in any other analysis. Animals were fasted for at least 4 hours, given one 3-gram meal, watched to ensure it was entirely eatenwithin 5 minutes and not provided additional food for at least 4 more hours. All other findings/analyses throughout the paper (circadian, ultradian, etc) are conducted in animals with ad libitum food.

4. Extended Data Fig. 5 is critical and should be included in the main part of the text. However, the legend poorly describes the figure and it is not clear what is going on. First of all, the acronyms CGM1, DT12, and flex1 should be included in the legend. Second, it is again unclear how the authors are analyzing ripple rate and delta current specifically for the meal. How long are the meals? Important question: to what extent is the negative correlation between SPW-R and future glucose concentrations stronger or weaker during various stages of activity (e.g., sleep vs. eating vs. awake but not eating vs. immediately postprandial, etc.)? This was not clearly elaborated on. The authors conclude that short duration SPW-Rs are

associated with thelargest decrease in future glucose concentrations, but from the way the data are presented and described, it is unclear to what extent the behavior of the animal (e.g., eating) influences this relationship. Part of my confusion relates to comment #2 above, as in many cases the figure legends do not do a good job of communicating the main result(s) of the figure.

The original acronyms were the animal's names but have been excluded from the current manuscript. The meals consisted of a single 3-gram pellet which was fully consumed within 5 minutes. Figure 2I-K now addresses the Reviewers questions regarding the magnitude of the SPW-R/glucose correlation and how it may change across fed-vs-fasting and sleep-vs-wake states. To summarize, eating does tend to increase the variability of this correlation during the postprandial period. However, the average peak of the correlation is similar to the fasting state.

1. The conditions for the optogenetics experiment in Fig. 6 are not clear, from either the Results or the Methods. Did the animals have access to food, or was food withheld? Also, the authors should include photomicrographs showing AAV injection sites for the ChR2.

Animals had *ad libitum* access to food/water for the entirety of the optogenetic experiments, similar to the correlational experiments (with the exception of data shown in Figure 2I-K for fed/fasting). These details have been added to the Results and Methods sections. We also haveincluded an example photomicrograph of the AAV injection site for both hippocampal and the new posterior parietal cortex optogenetic experiments (Extended Figure 12).

2. The lateral septum data were interesting, but then nothing was followed-up on. Is LS activityalso related to interstitial glucose concentrations? Would LS lesions (or inactivation) block the relationship between CA1d SPW-R and glucose?

We dedicated a novel experiment to address the Reviewer's critique. This involved bilateral LS injection of AAV1-hDlx-GiDREADD-dTomato-Fishell allowing for the pharmacogenetic inactivation of LS neurons with CNO. These experiments revealed that during suppression of LSneuronal activity, the correlation between SPW-Rs and glucose fluctuation was reduced to chance levels (Figure 5). This loss of function perturbation experiment provides further support for the role of the hippocampus in controlling peripheral glucose fluctuation, specifically through the HPC-LS circuit.

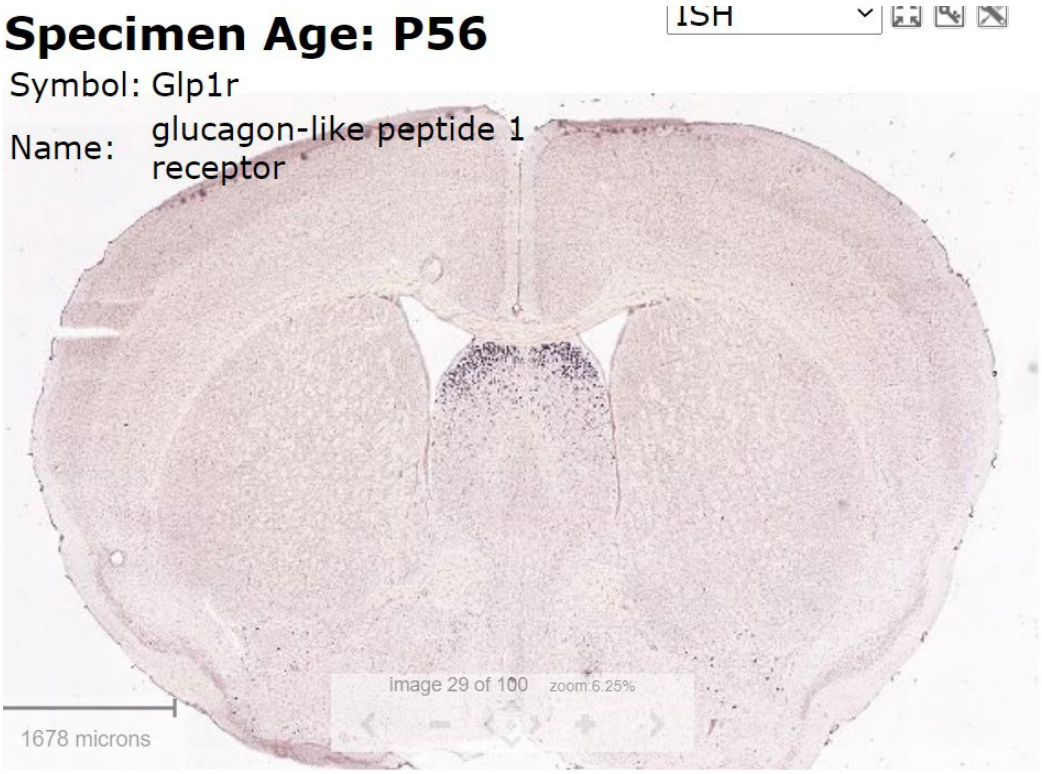
3. Related to #6, it was not clear exactly why the authors chose dorsal CA1 and the LS as targets of interest in the first place (vs. other hippocampal regions, and/or other downstream targets). They mention that the LS is "the major relay nucleus between hippocampal-subicular- entorhinal system and the hypothalamus". However, the ventral CA1 has direction projections toBOTH the LS and the hypothalamus (e.g., lateral region).

This is an important point. To respond to the Reviewer's critique, we performed a new experiment in which we recorded SPW-Rs from both the dorsal segment and the ventral tip of the hippocampus simultaneously (Extended Data Figure 13). In doing so, we were able to replicate previous work showing that dorsal and ventral SPW-R occurrence are largely independent ((Sosa et al., 2020); Extended Data Figure 13D). Additionally, we found that SPW-Rs from dorsal CA1 more strongly correlated with peripheral glucose levels (Extended Data Figure 13E, F).

Indeed, vHPC has direct projections to the hypothalamus. On the other hand, LS receives dense inputs from the entire hippocampus, subiculum and entorhinal cortex (Risold and Swanson, 1997) and targets various hypothalamic nuclei. Our experiments do not exclude the potential complementary role of the vHPC-hypothalamic projection. On the other hand, the optogenetic and the new LS inactivation experiments provide solid support for our main conclusion that hippocampal SPW-Rs are instrumental in decreasing glucose levels in the body.

An additional argument in favor of our choice of the lateral septum is that several studies have now found that its dorsal region expresses the highest level of GLP1 receptors in the entire brain. Activating these receptors can alter dLS excitability [see figure below taken from Allen Institute ISH data, and (Harasta et al., 2015; Terrill et al., 2016, 2019)]. Both glucagon and GLP1 can bind this receptor (glucagon has a lower affinity (Runge et al., 2003)), and it has alsobeen shown that glucagon-like peptide (GLP1) and glucagon both cross the blood-brain barrier (Banks and Kastin, 1985; Kastin et al., 2002). The emerging view from these studies is that dLSexcitability may be modulated by peripheral fluctuations in GLP1 and glucagon, thus changing how dLS responds to hippocampal inputs. A potential prediction from this observation is that HPC-LS coupling strength during SPW-Rs (Tingley and Buzsáki, 2020) may be hormone-level dependent, and in turn alter the ability of SPW-Rs to reach peripheral metabolic effectors, whichmay itself be under hormonal regulation.

This is particularly interesting given the effect of CNO in our DREADD experiments correlated most strongly with dorsal lateral septal viral expression (R = 0.77 in dLS versus R = 0.3 in vLS;Extended Data Figure 14C, D).

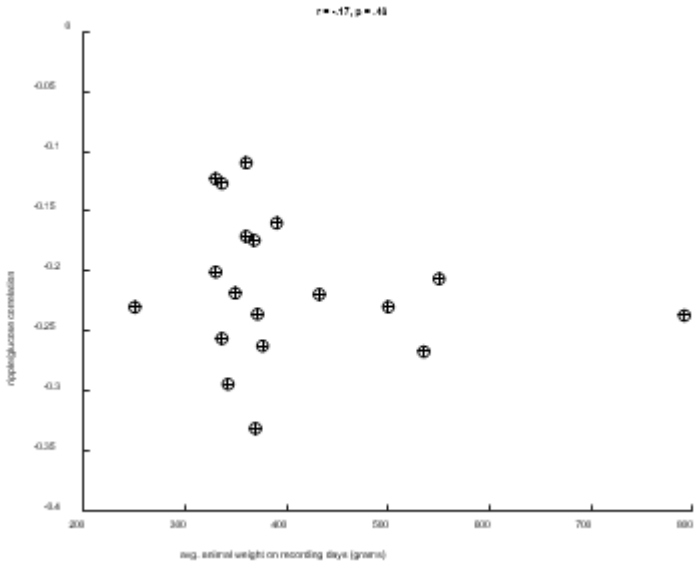


1. The authors hypothesize that their results may relate to the connection between sleep quality and T2 diabetes and/or obesity. This is clearly a testable hypothesis that would greatly

enhancethe paper: is the hippocampal SPW-R – glucose relationship altered in diabetic and/or obese rats.

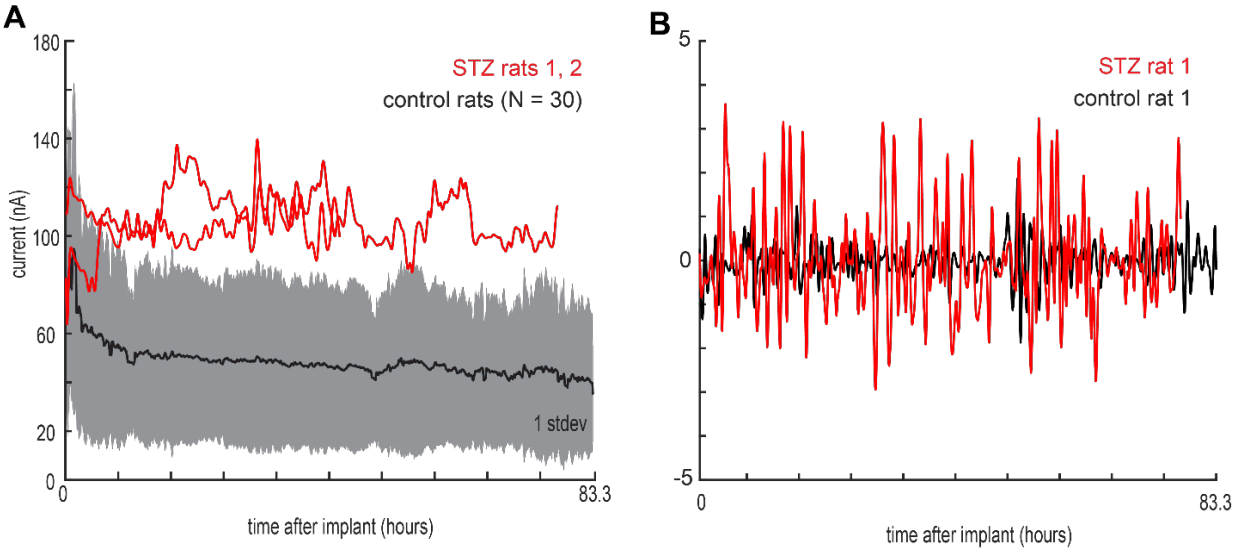
We strongly agree with the Reviewer that the suggested experiments will be enormously helpfultranslationally. The present experiments with its numerous correlational and perturbation experiments form the foundation for these next set of experiments, which will be very exciting intheir own right. In an attempt to address the Reviewer's suggestion, we increased the number ofrats and the range of weights over which animals underwent this experiment. Across the typical range of weights that are expected from adult rats, we did not observe a significant correlation between these weights and the correlation between SPW-R rate and glucose fluctuations.

Weights were averaged across recording days (typically 4-5 continuous days; see figure below).It should be noted that these animals were not presented with any kind of altered diet and that age is a major co-variate of the weights reported. During normal ('physiological') aging, associated with steady increase of bodyweight, SPW-R incidence does not increase, as long asit is normalized by immobility and sleep time, although SPW-R frequency slows down (Cowen etal., 2020). However, it is quite possible that in diabetic animals with their altered metabolic mechanisms, both sleep and SPW-R alternations will be found.



In a more direct attempt to address the Reviewer's comments (and that of Reviewer 1), we triedsuch an experiment. In 3 rats, we pharmacologically destroyed insulin-producing cells in the pancreas by streptozotocin injection. This is a standard diabetes rodent model (Wei et al., 2003). Our naïve working hypothesis was that after elimination of the beta cells of the pancreas, that the peripheral glucose rhythm which is so prominent at the 60-minute scale (Fig. 1B; Extended Data Figure 2D-E) would no longer be present for SPW-Rs to phase reset(Fig. 2H). The procedure worked effectively as shown by the two to three-fold increase of absolute glucose levels (glucose levels >250mg/dL). However, to our surprise and disappointment, the 60-minute fluctuation, did not disappear but, instead, was enhanced substantially (panel B below; avg. standard deviations were 1.43 nA vs 0.39 nA). This finding tells us that multiple peripheral factors (potentially including glucagon fluctuations and/or liver

mechanisms) can support the glucose rhythms whose peripheral regulation needs to be better understood in future experiments. There may be other ways to eliminate the glucose fluctuation (such as glucose clamp or denervation of peripheral organs) but such methods are beyond our current abilities.



(A) Current readings from the iPro2 device relative to the amount of time after placement. Black line is the average current readings from 30 intact rats, showing the slow decrease which corresponds to enzyme (glucose oxidase) breakdown on the electrode. Red lines are two STZ animals, showing glucose levels

~2.5-fold higher that control animals. **(B)** The variability in glucose levels was substantially larger in STZanimals.

Referee #3 (Remarks to the Author):

Tingley et al. investigate correlation between sharp wave ripple rate and interstitial glucose concentration in rats with the view of assessing whether hippocampal SWRs may also support ageneral metabolic function. The authors find periods characterised by high (>100 rip/5min) SWRrate are associated with low glucose concentration. Further, the authors show this negative relationship between SWR rate and glucose concentration can be artificially reinstated by optogenetically inducing SWRs. Finally, although circadian modulation of SWR rate and glucoseconcentration is anti-correlated (periods of high glucose concentration during a 24hr light cyclesare associated with a lower SWR rate (fig2E, fig3c)) the authors claim the relationship between SWR rate and glucose concentration cannot be merely accounted for by vigilance state (NREM vs awake). These findings are undeniably novel and if true then this would challenge traditional theories of SWR function and could lead to a novel research direction.

However, I remain to be convinced the relationship between SWR rate and glucose levels is notjust a result of changes in vigilance/brain state. The paper's conclusions would benefit from a number of further analyses to eliminate this alternative explanation. Moreover, more informationabout their glucose measurement and state scoring method is required. I outline each of my concerns below.

We agree with the Reviewer that disentangling correlated brain/body states from mechanistic

drivers is a difficult problem. As outlined in our responses below, we have tried our best to satisfy the Reviewer's (and our own) skepticism.

- It seems the majority (>75%) of SWRs occur during NREM sleep, however could the authors show the relationship between glucose concentration and SWR rate is maintained if only awakeSWRs are analysed? If it is, this would provide a more convincing evidence that SWR rate x glucose correlation is not just mediated by brain state (e.g. NREM sleep).

We thank the Reviewer for pointing out that this distinction was not apparent in our original manuscript. Please see the new Figure 2L-N, where we have separately examined periods ofNREM and waking, and found virtually identical relationships between SPW-Rs and glucose levels during these brain states. We also observed no difference in correlation between fed/fasting states where vigilance/motivational states are quite different (Figure 2I-K).

- Could the authors differentiate between different stages of NREM sleep. Rats, similar to humans, go through different stages of NREM sleep (e.g. see Lacroix et al. (2018). High ripplerates may reflect deep NREM sleep specifically (as indeed mentioned by the authors in the discussion). Thus, perhaps the SWR rate x glucose correlation can be explained by depth of NREM. The authors need to address this confound.

The Reviewer is right, and we apologize for the lack of clarity here. We employed several continuous metrics in the GLM predictions of glucose dynamics (now Figure 3), specifically to capture different stages/variability within a given brain state. The power spectrum slope (PSS) isa metric that differentiates second-by-second changes in brain state rather than binarized (Sleep/wake) differentiations. As such, it captures quite well the depth of NREM sleep (Levenstein et al., 2019). Please note in Figure 3 that PSS and theta/delta ratio were the two worst predictors of glucose fluctuations, compared to SPW-Rs. These findings, together with ouroptogenetic experiments, imply that it is not simply sleep depth but the pulsatile change associated with SPW-Rs that is what matters because, as we hypothesize, such a pulsatile signal may reset the peripheral glucose rhythm. It can also be mentioned here that the optogenetic induction of ripples did not correlate with any of the brain/body states which we were able to measure (Extended Data Figure 11).

- Related to this, the methods section on sleep scoring was very brief. It is not clear how the authors are differentiating between NREM and awake states. Further, NREM vs awake is a verycrude distinction and perhaps is why the awake/nrem predictor in their model is not able to capture as much of the variance in glucose levels as SWR rate.

The question of the Reviewer is an ever-recurring issue in sleep scoring. While traditionally the human EEG is divided into stages (originally four stages of NREM and REM, more recently onlythree stages of NREM, REM and microarousals), most sleep researchers agree that boundariesare fuzzy. For these reasons, several contemporary methods use various continuous metrics.
We adopted PSS (i.e., the slope of the power spectrum) because it has been shown to quantifysleep-wave-active wake stages well by a single quantity in both humans (Donoghue et al., 2020) and rodents (Levenstein et al., 2019). In addition, we also use more traditional metrics like the theta residual (i.e., power) and theta/delta ratio.

- Although the authors show inducing ripples also leads to a reduction in glucose, this effect is substantially weaker than that found for spontaneous ripples (e.g. if you compare fig4F and 6E).Moreover, inducing ripples at a high rate may affect oscillatory activity in down-stream cortical networks. Could the authors show synthetic ripple clusters do not lead to NREM-like cortical oscillations (e.g. delta waves).

Indeed, the induced ripple effect was weaker than the correlation between physiologically occurring ripples and peripheral glucose level. Yet, we were pleasantly surprised at the relatively high correlation of our artificial perturbation (peri-SPW-R: 0.094 nA; peri-stimulation:
0.059 nA). It was almost as strong as in control animals for 6 of the 8 rats used for the optogenetic experiment. This is surprising given that optogenetically induced ripples

remain mainly local (Stark et al., 2015), activating only a segment of the entire hippocampus, whereas physiological SPW-Rs can arise across a much larger population of neurons. Thus, theoptogenetic experiments give credence to the hypothesis that SPW-Rs in the dorsal hippocampus are indeed involved in the transient dips of glucose concentration.

We agree that individual CA1 stimulations may reach neocortex as it is the second most denseprojection pathway. However, optogenetic induction of ripples in dorsal CA1 did not alter overallbrain state in a manner that was detectable (NREM-like delta waves would have been detectedwith both the PSS and Wake/NREM ratio; Extended Data Figure 11B, E). Additionally, our newcontrol experiment showed that stimulation of the posterior parietal cortex was unable to drive glucose decreases (Extended Data Figure 12).

- The description of the glucose concentration measurement method is very brief. Could the authors provide more details here, for example the sampling rate of glucose levels is not given.

We have expanded this section of the

methods.Minor comments:
- There are a number of typos in the figures and legend. e.g. fig1c - one line is meant to be blue
and the other red, but currently both are red (making it very hard to interpret the figure). Thereare similar errors in other figure panels in the manuscript.

We have corrected these and several other typos as well as carefully edited the revised versionof the manuscript.

References

Abrams, R.M., Hutchison, A.A., Jay, T.M., Sokoloff, L., and Kennedy, C. (1988). Local cerebral glucose utilization non-selectively elevated in rapid eye movement sleep of the fetus. Dev. BrainRes. *40*, 65–70.

Aghakhani, Y., Bagshaw, A.P., Bénar, C.G., Hawco, C., Andermann, F., Dubeau, F., and Gotman, J. (2004). fMRI activation during spike and wave discharges in idiopathic generalizedepilepsy. Brain *127*, 1127–1144.

Attwell, D., and Gibb, A. (2005). Neuroenergetics and the kinetic design of excitatory synapses.Nat. Rev. Neurosci. *6*, 841–849.

Banks, W.A., and Kastin, A.J. (1985). Peptides and the blood-brain barrier: Lipophilicity as apredictor of permeability. Brain Res. Bull. *15*, 287–292.

Braun, A.R., Balkin, T.J., Wesensten, N.J., Carson, R.E., Varga, M., Baldwin, P., Selbie, S., Belenky, G., and Herscovitch, P. (1997). Regional cerebral blood flow throughout the sleep-wake cycle. An H215O PET study. Brain *120*, 1173–1197.

Buxton, O.M., Pavlova, M., Reid, E.W., Wang, W., Simonson, D.C., and Adler, G.K. (2010). Sleep restriction for 1 week reduces insulin sensitivity in healthy men. Diabetes *59*, 2126–2133.Buzsáki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memoryand planning. Hippocampus *25*, 1073–1188.

Buzsáki, G., Horvath, Z., Urioste, R., Hetke, J., and Wise, K. (1992). High-frequency networkoscillation in the hippocampus. Science (80-.). *256*, 1025–1027.

Cappuccio, F.P., D'Elia, L., Strazzullo, P., and Miller, M.A. (2010). Quantity and quality of sleepand incidence of type 2 diabetes: A systematic review and meta-analysis. Diabetes Care *33*, 414–420.

Cowen, S.L., Gray, D.T., Wiegand, J.P.L., Schimanski, L.A., and Barnes, C.A. (2020).

Age-associated changes in waking hippocampal sharp-wave ripples. Hippocampus *30*, 28–38.

Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A., and Buzsáki, G. (1999). Fast network oscillations in the hippocampal CA1 region of the behaving rat. J. Neurosci. *19*, RC20.
Donga, E., Van Dijk, M., Van Dijk, J.G., Biermasz, N.R., Lammers, G.J., Van Kralingen, K.W.,
Corssmit, E.P.M., and Romijn, J.A. (2010). A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. J. Clin. Endocrinol. Metab.*95*, 2963–2968.
Donoghue, T., Haller, M., Peterson, E.J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A.H., Wallis, J.D., Knight, R.T., et al. (2020). Parameterizing neural power spectra into periodicand aperiodic components. Nat. Neurosci. *23*, 1655–1665.
English, D.F., Peyrache, A., Stark, E., Roux, L., Vallentin, D., Long, M.A., and Buzsáki, G.(2014). Excitation and inhibition compete to control spiking during hippocampal ripples: Intracellular study in behaving mice. J. Neurosci. *34*, 16509–16517.
Gangwisch, J.E., Heymsfield, S.B., Boden-Albala, B., Buijs, R.M., Kreier, F., Pickering, T.G.,Rundle, A.G., Zammit, G.K., and Malaspina, D. (2007). Sleep duration as a risk factor for diabetes incidence in a large US sample. Sleep *30*, 1667–1673.
Gold, P.E. (2005). Glucose and age-related changes in memory. Neurobiol. Aging *26*, 60–64. Güemes, A., and Georgiou, P. (2018). Review of the role of the nervous system in glucose homoeostasis and future perspectives towards the management of diabetes. Bioelectron. Med.*4*, 1–18.
Harasta, A.E., Power, J.M., Von Jonquieres, G., Karl, T., Drucker, D.J., Housley, G.D., Schneider, M., and Klugmann, M. (2015). Septal glucagon-like peptide 1 receptor expressiondetermines suppression of cocaine-induced behavior. Neuropsychopharmacology *40*, 1969–1978.
Hughey, C.C., Hittel, D.S., Johnsen, V.L., and Shearer, J. (2010). Hyperinsulinemic-euglycemicclamp in the conscious rat. J. Vis. Exp. 1–6.
Kastin, A.J., Akerstrom, V., and Pan, W. (2002). Interactions of Glucagon-Like Peptide-1 (GLP-1) with the Blood-Brain Barrier. *18*, 7–14.
Korol, D.L., and Gold, P.E. (1998). Glucose, memory, and aging. Am J Clin Nutr *67*, 764–771. Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., et al. (2007). Genome-wide atlas of gene expression in theadult mouse brain. Nature *445*, 168–176.
Leproult, R., and Van Cauter, E. (2010). Role of Sleep and Sleep Loss in Hormonal Releaseand Metabolism. Endocr Dev *17*, 11–21.
Levenstein, D., Buzsáki, G., and Rinzel, J. (2019). NREM sleep in the rodent neocortex andhippocampus reflects excitable dynamics. Nat. Commun. *10*, 1–12.
Maquet, P. (2000). Functional neuroimaging of normal human sleep by positron emissiontomography. J. Sleep Res. *9*, 207–231.
Nedeltcheva, A. V., Kessler, L., Imperial, J., and Penev, P.D. (2009). Exposure to recurrent sleep restriction in the setting of high caloric intake and physical inactivity results in increasedinsulin resistance and reduced glucose tolerance. J. Clin. Endocrinol. Metab. *94*, 3242–3250.Risold, P.Y., and Swanson, L.W. (1997). Connections of the rat lateral septal complex. Brain Res. Rev. *24*, 115–195.
Runge, S., Wulff, B.S., Madsen, K., Bräuner-Osborne, H., and Knudsen, L.B. (2003). Different domains of the glucagon and glucagon-like peptide-1 receptors provide the critical determinantsof ligand selectivity. Br. J. Pharmacol. *138*, 787–794.
Schmid, S.M., Hallschmid, M., Jauch-Chara, K., Wilms, B., Benedict, C., Lehnert, H., Born, J.,and Schultes, B. (2009). Short-term sleep loss decreases physical activity under free-living conditions but does not increase food intake under time-deprived laboratory conditions in healthy men. Am. J. Clin. Nutr. *90*, 1476–1482.
Schmid, S.M., Hallschmid, M., Jauch-Chara, K., Wilms, B., Lehnert, H., Born, J., and Schultes,

B. (2011). Disturbed glucoregulatory response to food intake after moderate sleep restriction.
Sleep *34*, 371–377.
Schmid, S.M., Hallschmid, M., and Schultes, B. (2015). The metabolic burden of sleep loss.Lancet Diabetes Endocrinol. *3*, 52–62.
Shimazu, T. (1987). Neuronal regulation of hepatic glucose metabolism in mammals. Diabetes.Metab. Rev. *3*, 185–206.
Sosa, M., Joo, H.R., and Frank, L.M. (2020). Dorsal and Ventral Hippocampal Sharp-WaveRipples Activate Distinct Nucleus Accumbens Networks. Neuron *105*, 725-741.e8.
Spiegel, K., Leproult, R., and Van Cauter, E. (1999). Impact of sleep debt on metabolic andendocrine function. Lancet *354*, 1435–1439.
Stark, E., Roux, L., Eichler, R., Senzai, Y., Royer, S., and Buzsáki, G. (2014). Pyramidal Cell-Interneuron Interactions Underlie Hippocampal Ripple Oscillations. Neuron *83*, 467–480.
Stark, E., Roux, L., Eichler, R., and Buzsáki, G. (2015). Local generation of multineuronal spikesequences in the hippocampal CA1 region.
Terrill, S.J., Jackson, C.M., Greene, H.E., Lilly, N., Maske, C.B., Vallejo, S., and Williams, D.L.(2016). Role of Lateral Septum Glucagon-Like Peptide 1 Receptors in Food Intake. Am. J. Physiol. - Regul. Integr. Comp. Physiol. ajpregu.00460.2015.
Terrill, S.J., Holt, M.K., Maske, C.B., Abrams, N., Reimann, F., Trapp, S., and Williams, D.L. (2019). Endogenous GLP-1 in lateral septum promotes satiety and suppresses motivation forfood in mice. Physiol. Behav. *206*, 191–199.
Tingley, D., and Buzsáki, G. (2020). Routing of Hippocampal Ripples to Subcortical Structuresvia the Lateral Septum. Neuron *105*, 138-149.e5.
Watson, B.O., Levenstein, D., Greene, J.P., Gelinas, J.N., and Buzsáki, G. (2016). NetworkHomeostasis and State Dynamics of Neocortical Sleep. Neuron *90*, 839–852.
Wei, M., Ong, L., Smith, M.T., Ross, F.B., Schmid, K., Hoey, A.J., Burstow, D., and Brown, L.(2003). The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. Hear. Lung Circ. *12*, 44–50.
Zanos, T.P., Silverman, H.A., Levy, T., Tsaava, T., Battinelli, E., Lorraine, P.W., Ashe, J.M., Chavan, S.S., Tracey, K.J., and Bouton, C.E. (2018). Identification of cytokine-specific sensoryneural signals by decoding murine vagus nerve activity. Proc. Natl. Acad. Sci. U. S. A. *115*, E4843–E4852.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have added a number of control experiments and analyses. Specifically, the finding that pharmacogenetic silencing of the lateral septum abolishes the correlation between SPW-Rs and glucose levels provides important information supporting the authors' claim for a regulatory role of SPW-Rs in peripheral glucose levels. I am also fully aware that currently multiple technical problems and restrictions may prevent the straightforward demonstration of a regulatory loop controlled by SPW-Rs. Thus, despite the authors' strong efforts two of my major points appear to be not fully addressed:

As to the question whether the decrease in glucose concentrations following SPW-Rs might be a globalized response to increased energy demands, they argue with respect to local spike activity, which overall remains unchanged around hippocampal SPW-Rs. This reasoning, however, appears to be a little at odds with findings in monkeys by Logothetis et al, Nature 2012 showing that SPW-

Rs triggered strong increases in BOLD signal that comprised most of the cerebral cortex, apart from hippocampus.

As to the putative glucose regulatory loop, I cannot see (in Extended Data Figure 7B and C) "a 'ringing' with a cycle time of ~60 minutes" that is truly exceeding noise levels. The (statistical) robustness of the data shown in Extended data 7E is difficult to assess. Overall no clear evidence for feedback regulation.

As to the new loss-of-function experiments after silencing LS (Figure 5), were these SPW-Rs also recorded during NREM sleep?

Referee #2 (Remarks to the Author):

The authors have sufficiently addressed my concerns, and in my opinion, the concerns of the other reviewers as well.

Author Rebuttals to First Revision:

Referee #1 (Remarks to the Author):

The authors have added a number of control experiments and analyses. Specifically, the finding that pharmacogenetic silencing of the lateral septum abolishes the correlation between SPW-Rs and glucose levels provides important information supporting the authors??? claim for a regulatory role of SPW-Rs in peripheral glucose levels. I am also fully aware that currently multiple technical problems and restrictions may prevent the straightforward demonstration of a regulatory loop controlled by SPW-Rs. Thus, despite the authors??? strong efforts two of my major points appear to be not fully addressed:

As to the question whether the decrease in glucose concentrations following SPW-Rs might be a globalized response to increased energy demands, they argue with respect to local spike activity, which overall remains unchanged around hippocampal SPW-Rs. This reasoning, however, appears to be a little at odds with findings in monkeys by Logothetis et al, Nature 2012 showing that SPW-Rs triggered strong increases in BOLD signal that comprised most of the cerebral cortex, apart from hippocampus.

We thank the Reviewer for re-emphasizing the potential confound/importance of brain energy cost related to SPW-Rs. Indeed, in the mentioned Logothetis et al., experiments it appeared that activity in the entire cortical mantle was increased, as detected by the BOLD signal. We and others have also found in rodents that several neocortical sites show an electrophysiological coupling with hippocampal SPW-Rs. The nature of this coupling though is rather complex. In the prefrontal cortex, SPW-Rs are followed by DOWN (silent) states, whereas in the entorhinal cortex and visual cortex, they are linked to an UP state. Initial findings in retrosplenial cortex suggest a rebalancing, where layer 5 is generally suppressed while layers 2/3 are activated during SPW-Rs. Importantly, the correlations are typically rather low (on the order of R = 0.1)[1]. Also, the 'causation' is bidirectional

such that some events in the neocortex are 'triggered' by SPW-Rs, whereas in many other cases the cortical events lead, rather than follow hippocampal SPW-Rs.

It should also be noted from the Logothetis et al. data, that the strongest SPW-R coupling to fMRI BOLD signal was primarily observed in *anesthetized* monkeys being given a large continuous intravascular glucose infusion. Coupling strengths were approximately halved when examined in unanesthetized animals, with many regions no longer being significantly coupled (V1, PFC; see Suppl. Figures 7 and 8 for this comparison).

Yet, we interpret the Reviewers' central concern to be that independent of the nature of the correlations, if the energy cost around SPW-Rs is high, that energy must be borrowed from some source, one of which could be blood delivered glucose. As far as we know there is no reliable estimation of this expected energy cost. We believe that our specifically designed manipulation clearly argues against this explanation. During pharmacogenetic inactivation of the lateral septum, the correlation between SPW-Rs and glucose levels was abolished. Since it is *unlikely that the lateral septum inactivation affected the neocortical responses and the postulated energy cost, this manipulation excludes or at least strongly reduces the possibility that the sole cause of our reported correlation* is nothing else but increased glucose utilization of cortical neurons during SPW-Rs. We added a sentence to the Discussion to address the Reviewer's comment ("The pharmacogenetic inactivation of LS also excludes an alternative interpretation of our data, namely that the glucose decrease in the periphery reflects direct glucose utilization by hippocampal-cortical networks due to SPW-R-related activity.").

Perhaps the closest body of work to the reviewer's question has to do with the relationship between the BOLD signal and **brain** glucose uptake rates. It appears that the brain-wide correlation between these two measurements is on the order of R=0.3 [2,3]. However, when this correlation is examined for specific brain regions, cortical (default mode) networks have correlations up to R=0.88, whereas limbic structures had weaker relationships, in some cases having negative correlations between these measurements (R=-0.11)[4]. Thus, to go from SPW-R associated spiking to brain-wide glucose uptake rate requires at least five conversion factors, 1) the metabolic cost of *synchronous* action potential firing, 2) hippocampal-neocortical spike coupling during SPW-Rs, 3) spike activity to BOLD coupling, 4) BOLD signal to FDG-PET signal coupling, and 5) coupling of the FDG-PET signal to physiological glucose uptake rates. Four of these relationships have been reported to be at most on the order of R=0.4 with regional and temporal variability, while the metabolic cost of fewer but more synchronous action potentials is currently unknown.

As to the putative glucose regulatory loop, I cannot see (in Extended Data Figure 7B and C) ???a ???ringing??? with a cycle time of ~60 minutes??? that is truly exceeding noise levels. The (statistical) robustness of the data shown in Extended data 7E is difficult to assess. Overall no clear evidence for feedback regulation.

The ringing, or oscillation, is visible in Fig. 2B, E, I, L, and Extended Fig. 2E. In Fig. 2E, the left positive peak is highly significant. Extended Fig. 7B and C are indeed noisier because these are examining only a fraction of large peaks or troughs, and we lose many data points. Yet, the SPW-R rate triggered on troughs of the glucose correlation (red) exceeds three standard deviations of the shuffled distribution at three different points in time (two negative cycles, one positive in between).

But perhaps the 60 min oscillation is visually more convincing in Extended Fig. 13E, where two sets of experiments from 3 (red) and 30 (grey) animals from dorsal hippocampal recordings are superimposed.

As to the new loss-of-function experiments after silencing LS (Figure 5), were these SPW-Rs also recorded during NREM sleep?

All data in these sessions were collected while the rat was in its home cage. The injections were done at 9am and the six-hour post-injection window is during their light (sleep) cycle. As such, most of the data are from NREM sleep or quiet wakefulness.

Referee #2 (Remarks to the Author):

The authors have sufficiently addressed my concerns, and in my opinion, the concerns of the other reviewers as well.

We thank the Reviewers again, as we feel that their feedback was highly constructive and lead to a more complete story upon revision.

1. Siapas, A. G. & Wilson, M. A. Coordinated Interactions between Hippocampal Ripples and Cortical Spindles during Slow-Wave Sleep. *Neuron* **21**, 1123–1128 (1998).
2. Wang, J. *et al.* The Relationship Among Glucose Metabolism, Cerebral Blood Flow, and Functional Activity: a Hybrid PET/fMRI Study. *Mol. Neurobiol.* **58**, 2862–2873 (2021).
3. Nugent, A. C., Martinez, A., D'Alfonso, A., Zarate, C. A. & Theodore, W. H. The relationship between glucose metabolism, resting-state fMRI BOLD signal, and GABA A -binding potential: A preliminary study in healthy subjects and those with temporal lobe epilepsy. *J. Cereb. Blood Flow Metab.* **35**, 583–591 (2015).

4. Aiello, M. *et al.* Relationship between simultaneously acquired resting-state regional cerebral glucose metabolism and functional MRI: A PET/MR hybrid scanner study. *Neuroimage* **113**, 111–121 (2015).

Reviewer Reports on the Second Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have satisfactorily addressed my remaining concerns in their rebuttal letter. As to the manuscript, I suggest that they more cautiously express their reasoning that findings after inactivation of the LS "exclude" that the glucose decrease in the periphery reflects direct glucose utilization by hippocampal-cortical networks due to SPW-R-related activity. It makes it unlikely but, cannot be completely ruled out. Also, the Logothetis et al. reference should be added to this sentence. As to my "feedback regulation" comment, this is important data and I suggest that the respective figures, i.e., Extended Fig. 7B and perhaps also Extended Fig. 13F are moved to the main text. With these two small changes the paper overall represents really awesome work!

Author Rebuttals to Second Revision:

N/A