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Supplementary information

A metabolic function of the hippocampal sharp wave-ripple

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Supplementary Information

Supplementary Table 1: List of animals used for experiments

rat ID	Project	surgery	Construct injected	Coordinates
rat 1	ripple/glucose rec	rCA1	NA	ap 4, ml 4, dv 1.5
rat 2	ripple/glucose rec	rCA1	NA	ap 4, ml 4, dv 1.5
rat 3	ripple/glucose rec	rCA1	NA	ap 4, ml 4, dv 1.5
rat 4	ripple/glucose rec	rCA1	NA	ap 4, ml 4, dv 1.5
rat 5	ripple/glucose rec	bilat CA1	NA	ap 4, ml 4, dv 1.5
rat 6	ripple/glucose rec	rHypothalamus & rCA1	NA	HYP: 2, 1.25, 2.3 & CA1 4, 3, 1.5
rat 7	ripple/glucose rec	rHypothalamus & rCA1	NA	HYP: 2, 1.25, 2.3 & CA1 4, 3, 1.5
rat 8	ripple/glucose rec	flex probe in rCA1	NA	ap 4, ml 4, dv 1.5
rat 9	Opto stim	bilat CA3 CaMKII-ChR2	AAV5-CamKII-ChR2-EYFP	ap 4, ml 4, dv 3.4 & 2.4
rat 10	Opto stim	bilat CA3 CaMKII-ChR2 (only @ 3.4mm)	AAV5-CamKII-ChR2-EYFP	ap 4, ml 4, dv 3.4
rat 11	Opto stim	bilat CA3 CaMKII-ChR2 (only @ 3.4mm)	AAV5-CamKII-ChR2-EYFP	ap 4, ml 4, dv 3.4
rat 12	Opto stim	bilat CA1 CaMKII-CHR2	AAV5-CamKII-ChR2-EYFP	ap 4, ml 3, dv 2.4 & 2
rat 13	Opto stim	bilat CA1 CaMKII-CHR2	AAV5-CamKII-ChR2-EYFP	ap 3.5, ml 2.5, dv 2.4 & 2
rat 14	Opto stim	bilat CA1 CaMKII-CHR2	AAV5-CamKII-ChR2-EYFP	ap 4, ml 3, dv 2.4 & 2
rat 15	Opto stim	bilat CA1 CaMKII-CHR2	AAV5-CamKII-ChR2-EYFP	ap 4, ml 3, dv 2.4 & 2
rat 16	Opto stim	bilat CA1 CaMKII-CHR2	AAV5-CamKII-ChR2-EYFP	ap 4, ml 3, dv 2.4 & 2
rat 17	dorsal/ventral	NA	NA	dors: -2.5AP, 2ML; vent: -4.9AP, 5ML
rat 18	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 19	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4, 4.6, 3.8DV
rat 20	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4, 4.6, 3.8DV
rat 21	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 22	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 23	dorsal/ventral	dorsal/ventral probe implant	NA	dors: -2.5AP, 2ML; vent: -4.9AP, 5ML
rat 24	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 25	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 26	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 27	DREADDS	LS injected	AAV1-hDlx-GiDREADD-dTomato-Fishell	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 28	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 29	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 30	DREADDS	MS injected	AAV2-hSyn-hM4D(Gi)-mCherry	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 31	dorsal/ventral	dorsal/ventral probe implant	NA	dors: -2.5AP, 2ML; vent: -4.9AP, 5ML
rat 32	DREADDS	MS injected	AAV2-hSyn-hM4D(Gi)-mCherry	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 33	dorsal/ventral	dorsal/ventral probe implant	NA	dors: -2.5AP, 2ML; vent: -4.9AP, 5ML
rat 34	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 35	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 36	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 37	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 38	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 39	PPC opto stim	PPC injected	AAV5-CamKII-ChR2-EYFP	-4 AP, 3ML, 0.8, 0.4 DV
rat 40	DREADDS	MS injected	AAV2-hSyn-hM4D(Gi)-mCherry	0.5 AP 0.5ML, 5.4,4.6,3.8DV
rat 41	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 42	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 43	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 44	DREADDS	CA1 implanted	NA	-4 AP, 3ML, 2.4, 1.5 DV
rat 45	ripple/glucose rec	CA1 implanted	NA	-4 AP, 3ML

Supplementary Discussion

Homeostatic and allostatic regulations represent a reciprocal loop with effector and sensor arms. Early studies demonstrated that electrical stimulation of the hippocampus elicits various hormonal responses, detected as fluctuations in growth hormone, glucocorticoid, glucose, and insulin concentrations in the blood stream[1–3]. Complementing this postulated effector directionality, there is abundant expression of hormonal receptors in the hippocampus[4,5], many of which affect hippocampal network excitability and cognitive functions[6–8]. Historically, there are two complementary viewpoints on body-brain interactions which involve hippocampal networks. One where hippocampal excitability plays a top-down effector role in affecting endocrine state, and a second where hippocampal excitability is itself modulated by hormonal state (feedback arm of the loop). We hypothesized that the hippocampal formation is one of the players in coordinating multi-organ allostatic and homeostatic control loops of metabolic state. However, the network conditions and activity patterns within the hippocampus that play a role in these hypothesized loops have remained undisclosed.

To accurately assess the potential relationship between SPW-Rs and metabolic fluctuations, we continuously monitored peripheral glucose concentration and recorded electrophysiological signals from the dorsal hippocampal CA1 region over extended time courses while animals lived normally in their home cages with *ad libitum* food and water, and a regular 12-hour light/dark cycle. Given the complexity of the postulated homeostatic system, and the fact that these systems behave qualitatively differently during stress, restraint, or anesthesia, we set out to perform these experiments in a freely behaving animal. In each animal, we validated that the shape, amplitude, and rate of hippocampal SPW-Rs were consistent across multiple days. On average, we observed 9.5 (± 8.2) SPW-Rs per minute (threshold >5 SD above background power in the 120-200 Hz band; or ~14,000 SPW-Rs per day) with a large degree of variance ranging from zero ripples for several minutes up to > 40 ripples in a minute (Figure 1F; Extended Data Figure 4). Comparison of SPW-R rate across day and night, long-term autocorrelograms, and spectral analysis of SPW-R rate fluctuations revealed that small portions of this variance could be explained by circadian, and ultradian mechanisms (Extended Data Figure 4).

As SPW-R rate and glucose metabolism are both modulated by food intake, we conducted an additional control experiment in four animals. Food access was restricted, and single 3-gram food pellets were provided at random times during the light cycle (10am-4pm). The correlation between SPW-Rs and glucose fluctuations was not different between fasting and fed states and was not modulated across this fasting-to-fed transition (Figure 2I-K; 36 meals in 4 rats). The correlation between SPW-Rs and glucose fluctuations was also consistent when subsets of the data were selected from different parts of the 24-hour circadian cycle or from different brain states (i.e. NREM/Waking; Extended Data Figure 4).

A caveat of cross-correlograms and peri-event time histogram analyses presented in Figure 2 is their inability to quantitatively isolate the contribution of multiple candidate predictors and to reveal co-modulation across multiple predictors. Therefore, we created a multivariate linear regression model that used all measured predictors to estimate glucose fluctuations. A second model was then constructed using all but one predictor ('leave-one-out' method). The mean squared error (MSE) for both models was computed and subtracted from each other for every temporal offset (±100 minutes) and for each withheld predictor (Extended Data Figure 6A). Thus, putative predictors that only add noise should have values centered near zero, while predictors that contribute non-redundant information about glucose fluctuations should have larger positive values. The largest MSE difference that we observed was for SPW-R rate at a positive 10-minute temporal offset (Extended Data Figure 6B).

A remaining caveat of this multivariate linear regression model is that the predictors used (SPW-R rate, theta power, movement, EMG, etc) may be correlated with each other, therefore the amount of variance explained can be 'split' between them. Therefore, in a second approach, only one predictor was used to train and test each model and the MSE values were compared in a pair-wise fashion across all candidate predictors (Extended Data Figure 6C). For all comparisons, SPW-R rate was a better predictor than other measured signals (Extended Data Figure 6D). SPW-R rate also persisted as the most informative predictor when using dimensionality reduction methods to search for latent variables which may correlate with multiple predictors (Extended Data Figure 6E, D). Given the degree of correlation between variables measuring

different aspects of the same internal state, we felt that these results, while suggestive, could not be solely relied upon when interpreting the role of SPW-Rs in glucose metabolism.

Therefore, we designed a 'gain-of-function' experiment where we selectively induced ripples in dorsal hippocampus. This protocol mimicked the dynamic range of naturally occurring SPW-Rs (0-40 per minute; Figure 1F, Extended Data Figures 3, and 7). While this experiment did replicate the negative correlation with glucose fluctuations, it could still have several mechanistic explanations. Given that SPW-Rs are known to be brain-wide events [9], an intuitive explanation could be that there is a heightened level of action potential firing in the neocortex during and after a SPW-R, resulting in a heightened metabolic cost in the brain, which could be reflected by a transient increase in the glucose uptake rate from the periphery. However, SPW-Rs are actually associated with a decrease in overall spiking rate when wider time windows with post-SPW-R silence are also considered (Extended Figure 3E). Additional experiments using the same stimulation protocol in posterior parietal cortex further suggest that the direct metabolic cost of stimulation is not mechanistically driving glucose concentration decreases (Extended Data Figure E-H). Most critically, LS inactivation eliminated the correlation between SPW-Rs and glucose (Figure 4), a manipulation that presumably left intact hippocampal-neocortical communication.

When examining the fluorescence expression patterns in the LS hM4Di animals, we found that this viral strategy often displayed additional expression within the medial septum (Extended Data Figure 10A). To address the question of specificity with regard to the lateral and medial septum, we utilized an additional viral construct (AAV2-hSyn-hM4Di-mCherry) which selectively targeted only the medial septum (Extended Data Figure 10B). In these animals, CNO injection had no effect on the correlation between SPW-R rate and glucose fluctuations (Figure 4; Extended Data Figure 10E-G. Taken together, pharmacogenetic inhibition of the lateral, but not medial, septum decoupled dorsal hippocampal SPW-Rs from peripheral glucose fluctuations of the body.

Given the present set of experiments, we submit that under physiological conditions, clusters of large amplitude hippocampal SPW-Rs provide a phase-resetting signal that reaches peripheral

organs, and in turn, phase-modulate glucose oscillations in the bloodstream and interstitial space. The organs most directly related to glucose homeostasis—the liver, pancreas, and adrenal gland—receive redundant signals from the autonomic nervous system as well as the hypothalamic-pituitary-adrenal (HPA) axis, allowing for allostatic predictions of future metabolic state[10,11]. Insulin and glucagon, the two major hormones to regulate glucose uptake and production, are not released continuously from the pancreas but in periodic, spatially synchronized bursts[12–14]. Importantly, these oscillatory bursts can be phase-reset by transient stimuli, such as carbachol infusion or vagus nerve stimulation[15,16].

While we cannot conclusively rule out the possibility of a hidden mechanism responsible for driving both SPW-Rs in the brain and glucose concentration changes in the periphery, 1) the reliability of this correlation across animals (Figure 2D), 2) the relative contribution of SPW-Rs among multiple brain and body signals when utilizing multivariate linear regression (Extended Data Figure 6), 3) the optogenetic induction of SPW-R events which drive a similar decrease in glucose concentrations (Figure 3D), and 4) the attenuation of this correlation by pharmacogenetic inhibition of the lateral septum (Figure 4D) all support the hypothesis that hippocampal SPW-Rs are effective in regulating peripheral glucose concentrations.

It is rare in nature to have effectors without any form of feedback. Insulin-sensitive glucose transporters and insulin receptors are more highly expressed in the hippocampal formation than in other cortical structures[5,17] and serve as a regulatory mechanism on glucose uptake and availability[17,18]. This presents the possibility for a negative feedback loop, as the direct action of insulin on the hippocampus has been shown to suppress network excitability[19,20], possibly regulating SPW-R occurrence. Additionally, glucagon-like peptide 1 receptors are highly expressed within the dorsal lateral septum where they modulate septal neuron excitability[21], and potentially alter the rectified-linear response to the impact of hippocampal SPW-Rs that we observe (Extended Data Figure 8). These forms of body-brain feedback may also explain why intrahippocampal infusion or intranasal application of insulin improves performance on working memory tasks[22–28] in a manner which is strikingly similar to the effects observed with direct manipulations of SPW-R occurrence[29–34].

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