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Food intake enhances hippocampal sharp wave-ripples

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eLife Assessment

This **important** study assessed the effects of food intake on sharp wave-ripples in the hippocampus of mice during subsequent sleep. **Convincing** evidence supports the conclusion that sharp wave-ripples are enhanced by food consumption. This work will likely interest researchers studying multiple functions including memory, metabolism, and brain-body physiology.

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Abstract

Effective regulation of energy metabolism is critical for survival. Metabolic control involves various nuclei within the hypothalamus, which receive information about the body's energy state and coordinate appropriate responses to maintain homeostasis, such as thermogenesis, pancreatic insulin secretion, and food-seeking behaviors. It has recently been found that the hippocampus, a brain region traditionally associated with memory and spatial navigation, is also involved in metabolic regulation. Specifically, hippocampal sharp wave ripples (SWRs), which are high-frequency neural oscillations supporting memory consolidation and foraging decisions, have been shown to reduce peripheral glucose levels. However, whether SWRs are enhanced by recent feeding– when the need for glucose metabolism increases, and if so, whether feeding-dependent modulation of SWRs is communicated to other brain regions involved in metabolic regulation, remains unknown. To address these gaps, we recorded SWRs from the dorsal CA1 region of the hippocampus of mice during sleep sessions before and after consumption of meals of varying caloric values. We found that SWRs occurring during sleep are significantly enhanced following food intake, with the magnitude of enhancement being dependent on the caloric content of the meal. This pattern occurred under both food-deprived and *ad libitum* feeding conditions. Moreover, we demonstrate that GABAergic neurons in the lateral hypothalamus, which are known to regulate food intake, exhibit a robust SWR-triggered increase in activity. These findings identify the satiety state as a factor modulating SWRs and suggest that hippocampal-lateral hypothalamic communication is a potential mechanism by which SWRs could modulate peripheral metabolism and food intake.

Introduction

For all organisms, from bacteria to humans, survival depends on searching for resources to maintain the internal environment in a desirable state. To obtain these resources, organisms perform stimulus-seeking behaviors wherein actions that improve bodily states are repeated while others are not. While even bacteria exhibit such coordination of behaviors based on the consequences of earlier actions (Keegstra et al., 2022), the evolution of the nervous system in more complex organisms has enabled the use of more sophisticated strategies to learn from the past, anticipate the future, and respond rapidly for efficient metabolic regulation.

In mammals, the hippocampus supports spatial navigation and the formation of episodic memories (O'Keefe & Nadel, 1978; Scoville & Milner, 1957), which is crucial for finding and recalling the locations of food sources. At the neurophysiological level, the hippocampus encodes the trajectory leading to food via the sequential firing of hippocampal place cells (O'Keefe & Nadel, 1978). During food consumption and subsequent resting periods, these same neurons replay the recent spatial trajectory in a temporally compressed manner during a highly synchronous oscillatory event called sharp wave-ripples (SWRs) (Buzsaki, 1989, 2015; Diba & Buzsaki, 2007; Joo & Frank, 2018; Skaggs & McNaughton, 1996; Wilson & McNaughton, 1994). SWRs communicate hippocampal activity patterns to the neocortex, supporting systems-level consolidation of memories (Brodt et al., 2023; Buzsaki, 2015; Klinzing et al., 2019).

In addition to the neocortex, the hypothalamus, a key regulator of homeostasis, receives dense multi-synaptic hippocampal innervation via the lateral septum (LS) (Risold & Swanson, 1997; Tingley & Buzsaki, 2020). Unlike the divergent hippocampo-cortical projections, the hippocampo-lateral septum projections are highly convergent, allowing the LS to detect only the magnitude of the SWR, but not the identity and temporal order of hippocampal neuronal spiking (Tingley & Buzsaki, 2020). Thus, the propagation of SWRs to hypothalamic nuclei is better suited to serve homeostatic functions rather than memory consolidation (Buzsaki & Tingley, 2023).

While earlier lesion and stimulation studies provided evidence that the hippocampus influences peripheral physiology and homeostatic behaviors (Clifton et al., 1998; Seto et al., 1983), direct physiological evidence demonstrating that SWRs serve metabolic functions only emerged recently. By combining *in vivo* electrophysiology with continuous glucose monitoring in freely behaving rats, Tingley and colleagues (2021) found a negative relationship between SWR rates and subsequent peripheral glucose levels. Moreover, they showed that optogenetically induced SWRs effectively reduced peripheral glucose levels, while chemogenetic inhibition of the LS abolished the correlation between SWR rate and glucose levels. Thus, this study strongly suggests that hippocampal SWRs serve a metabolic function.

The hypothesis that hippocampal SWRs have a metabolic function raises testable predictions regarding the relationship between SWRs and food consumption. The levels of glucose, the body's primary energy source, are tightly regulated by the nervous system (Lopez-Gambero et al., 2019). Specifically, around meal times, neuronal systems involved in glucose metabolism proactively and reactively coordinate the bodily response to the postprandial spike in glucose levels (Chen et al., 2015; Steculorum et al., 2016; Woods et al., 2006). Given that SWRs reduce peripheral glucose levels (Tingley et al., 2021), we set out to test the hypothesis that SWRs are enhanced following meal times as part of the systems-level response to increased glucose levels.

Results

To determine whether food consumption modulates SWRs during subsequent sleep, we recorded the local field potential (LFP) from the dorsal CA1 region of the hippocampus, along with electroencephalographic (EEG) and electromyographic (EMG) signals, in food-restricted mice during sleep sessions before and after food intake (**Fig. 1A-B**). Similar to previous studies investigating learning-dependent changes in hippocampal dynamics (Grosmark & Buzsaki, 2016; Oliva et al., 2020; Rothschild et al., 2017), our experimental procedure consisted of three sessions: a 2-hour sleep session ("Pre-sleep"), a subsequent food consumption session of roughly 1-hour ("Meal"), and an additional 2-hour sleep session ("Post-sleep"). Within the sleep sessions, the ongoing sleep stages were determined (Wake, REM and NREM, see Methods), and only NREM periods were included in further SWR analysis. There was no significant difference in the amount of time spent in NREM sleep in the Pre and Post sessions (P=0.1759, paired t-test, Suppl. Fig. 1). During the Meal sessions, mice received varying amounts of chow (0.3-1.5 gr) in their home cage without being subjected to any instructive learning paradigm. Given that mice typically consume 4-5 gr of chow per day (Bachmanov et al., 2002), the largest amount corresponded to about one-third of their daily food intake. In the control condition, the Meal session was replaced with a waking session of similar duration with no food delivery ("No-food"). Each mouse was tested in the Meal condition 3-12 times with varying amounts of chow and at least once in the No-food condition.

To assess whether food intake modulates SWRs, we calculated the fold change in SWR statistics from Pre-sleep to Post-sleep in paired sessions. We found that consuming larger meals (0.5-1.5 gr) significantly increased the ripple power of SWRs in all 38 session pairs, whereas smaller meals and the No-food condition did not result in a similar effect (**Fig. 1C**). We observed a similar modulation in ripple rate, which was increased in 31 of 38 sessions following consumption of larger meals (**Fig. 1D**), but not in ripple duration (**Fig. 1E**). Smaller meals and No-food condition did not induce a consistent change in ripple rate or duration (**Fig. 1D-E**). We then examined whether there is a relationship between meal size and the magnitude of change in ripple attributes. We found that the magnitude of increase in ripple power and rate, but not duration, was positively correlated with meal size (**Fig. 1F-H**). These findings suggest that the power and rate of SWRs increase following feeding in a dose-dependent manner.

Since the mice in the aforementioned experiments were food restricted, we wondered whether the observed increase in ripple power after large meals might be an indirect consequence of reduced sleep quality during hunger and its restoration following feeding (Goldstein et al., 2018). As a measure of non-rapid eye movements (NREM) sleep quality, we quantified cortical EEG delta power, which is also associated with SWR occurrence (Goldstein et al., 2018; Molle et al., 2006; Sirota et al., 2003). While delta power indeed increased after food intake in a dose-dependent manner (**Fig. 1I**), and the change in delta power was correlated with the change in ripple power (**Fig. 1J**), mediation analysis revealed that the effect of meal size on ripple power remained significant even after accounting for changes in delta power (**Fig. 1K**). Thus, while food intake enhanced delta power during sleep, it also led to a significant increase in ripple power beyond this effect.

To further rule out the possibility that recovery from hunger accounts for the observed increased ripple power following feeding, we conducted an additional set of experiments under *ad libitum* feeding conditions to ensure that sleep quality in the Pre-sleep session was not affected by hunger. To encourage eating during the Meal session under *ad-libitum* conditions, we replaced the standard chow with chocolate (0.1-1 gr) (**Fig. 2A**). Consistent with our previous findings, we found that chocolate consumption increased ripple power in subsequent sleep, with larger amounts of chocolate leading to greater increases in ripple power (**Fig. 2B**). Moreover, the

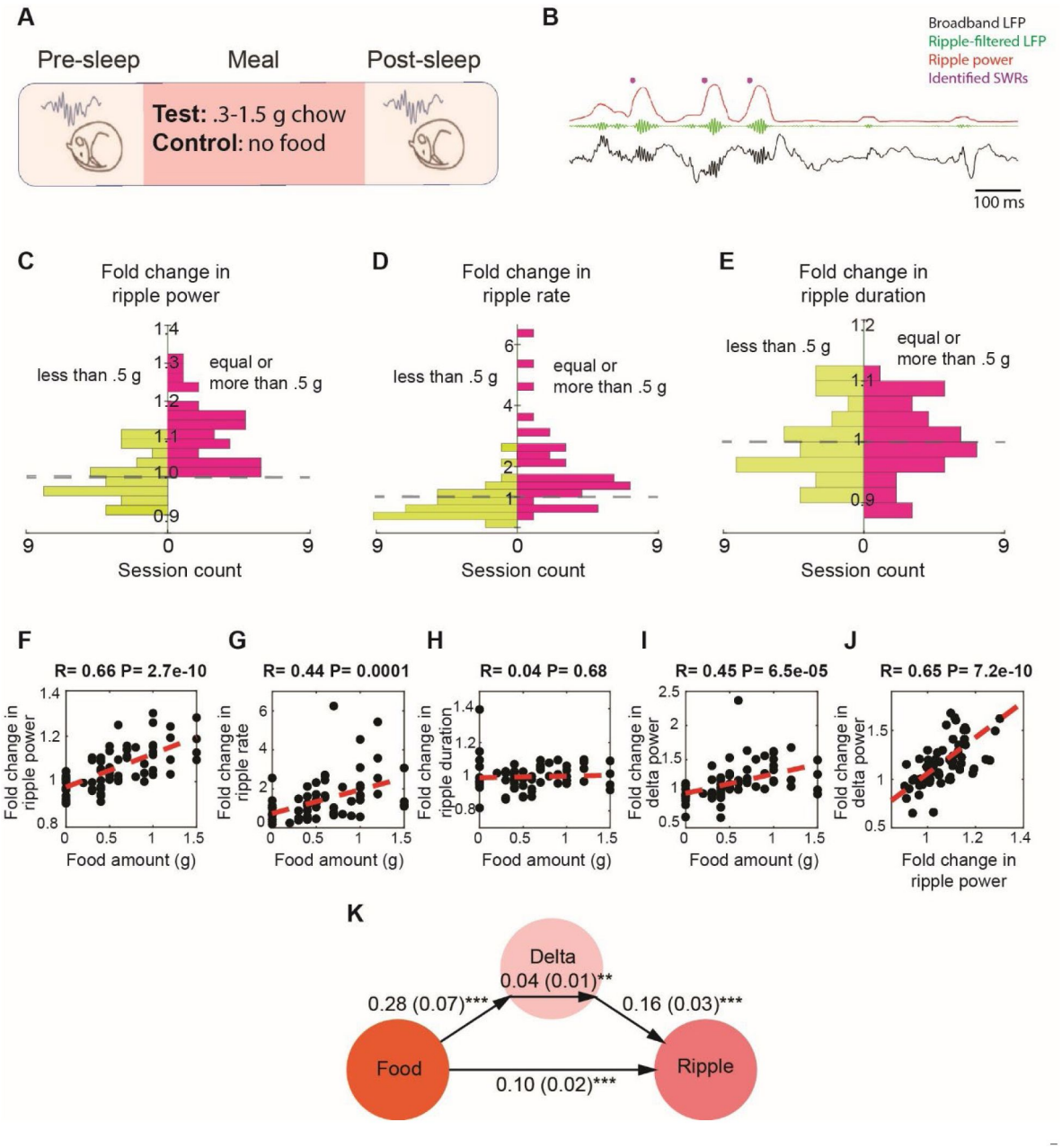

Figure 1.

Food intake enhances SWRs in sleep in a dose-dependent manner.

A. Experimental design **B**. A representative recording showing broadband hippocampal local field potential (LFP) (black), ripple-filtered LFP (150-250 Hz, green), ripple power (red) and detected SWRs (purple dots). **C-E.** The effect of chow intake on ripple power (C), ripple rate (D) and ripple duration (E). Red bars represent the changes observed following delivery of equal or more than 0.5 gr of chow, while the green bars represent the changes observed following delivery of less than 0.5 gr. **F-I**. Correlations between food amount and fold change in ripple power (F, R=0.66, P < 0.001), fold change in ripple rate (G, R=0.44, P < 0.001), fold change in ripple duration (H, NS) and fold change in delta power (I, R=0.45, P < 0.001). **J.** Correlation between fold change in ripple power and fold change in delta power (R=0.65. P < 0.001). Panels F-J include both the experimental and control animals-the control animals appearing as having received 0 food amount. **K.** A mediation analysis, indicating that the effect of food intake on ripple power was significant after accounting for sleep-dependent effects (correlation between the changes in delta power and ripple power: B = .16, SE = .03, p < .001; the effect of meal size on the change in delta power: B = .28, SE = .07, p < .001; the indirect effect of the change in delta power mediating the relationship between meal size and ripple power: B = .04, SE = .01, p < .05, the direct effect of meal size on the change in ripple power: B = .10, SE = .02, p < .001).

correlation between chocolate consumption and change in delta power was not significant, nor was the correlation with ripple rate or duration (**Fig. 2C-E**). Further mediation analysis revealed that the change in delta power did not significantly mediate the relationship between chocolate amount and the change in ripple power (**Fig. 2F**). Together, these results demonstrate that food intake enhances SWRs during sleep, even after accounting for the positive effect of food intake on sleep depth.

Next, we sought to uncover the mechanisms by which food intake modulates SWRs. Previous studies suggested that neural systems regulating food intake are responsive to both sensory and motor experiences related to feeding, such as food detection, chewing, and mechanical stretch of the gut, as well as humoral signals indicating energy availability (Bai et al., 2019; Chen et al., 2015; Thanarajah et al., 2019). To disentangle the factors underlying the meal-enhancement of SWRs, we first tested the effects of the caloric content of meals on SWRs by comparing the effects of caloric (1 gr of regular Jello) and non-caloric (1 gr of sugar-free Jello, containing artificial sweeteners) foods with similar sensory qualities (**Fig. 3A**). We reasoned that if their effects on SWRs differ, this would indicate a calorie-dependency in the meal-enhancement of SWRs, whereas no difference would suggest an experience-dependency. We found that consumption of regular Jello, but not sugar-free Jello, resulted in an increase in ripple power and ripple rate compared to the No-food condition (**Fig. 3B-C**). Additionally, the increase in ripple power, but not rate, was higher following consumption of regular Jello compared to sugar-free Jello (**Fig. 3B-C**). No differences were found in ripple duration or cortical delta power across conditions (**Fig. 3D-E**). These results suggest that the caloric content of meals, rather than the oro-/mechano-sensory experience of eating, underlies the post-meal enhancement in SWRs.

We next tested whether feeding-associated hormones contribute to the meal-enhancement of SWRs (**Fig. 4A**). Ghrelin, a prominent gut hormone, rises during fasting (Toshinai et al., 2001) and promotes increases in glucose levels and food intake (Poher et al., 2018). In contrast, meals trigger the release of insulin, glucagon-like peptide-1 (GLP-1), and leptin, which lower glucose levels and induce satiety (Gutzwiller et al., 1999; Woods et al., 2006). To investigate whether changes in these hormone concentrations modulate SWRs, we administered GLP-1, leptin, insulin and ghrelin to mice at different doses under *ad libitum* and food restricted conditions. Administration of GLP-1, leptin and insulin did not produce robust, consistent and dose-dependent effects on SWR attributes (**Fig. 4B-F**). On the other hand, ghrelin administration resulted in a dose-dependent decrease in ripple rate under ad libitum conditions (**Fig. 4H**) and a decrease in ripple power under food-restricted conditions (**Fig. 4I**). While ghrelin administration also reduced cortical delta power, mediation analysis revealed that the effect of ghrelin on ripple power remained significant even after accounting for change in cortical delta power under food-restricted conditions (**Fig. 4Ii-ii**). These results suggest that ghrelin has a dose-dependent negative effect on ripple rate and power, which exceeds its influence on sleep quality. In contrast, we did not find consistent modulation of SWRs following GLP-1, insulin, or leptin administration, despite their known effects on hippocampal memories and synaptic transmission (Abbas et al., 2009; McNay et al., 2010; Oomura et al., 2006). It is possible that specific aspects of our experimental design (e.g., focusing on the 2-hour Post-sleep period, administering only one hormone at a time, or using subcutaneous delivery) may have occluded potential effects of these hormones on SWRs.

Lastly, as our findings reveal that SWRs are modulated by food-related signals, we tested the hypothesis that SWRs can, in turn, influence brain regions involved in feeding regulation. Among the hypothalamic nuclei that participate in feeding regulation (Anand & Brobeck, 1951; Stuber & Wise, 2016), the lateral hypothalamus (LH) receives the densest di-synaptic input from the dorsal hippocampus via the lateral septum (Risold & Swanson, 1997; Tingley & Buzsaki, 2020). To test whether SWRs affect neuronal activity in the LH, we combined LFP recordings from the hippocampus with optical recordings from GABAergic neurons in the LH using fiber photometry. We used Vgat-IRES-Cre mice (n = 5) and transduced LH neurons with the pGP-AAV5-syn-FLEX-

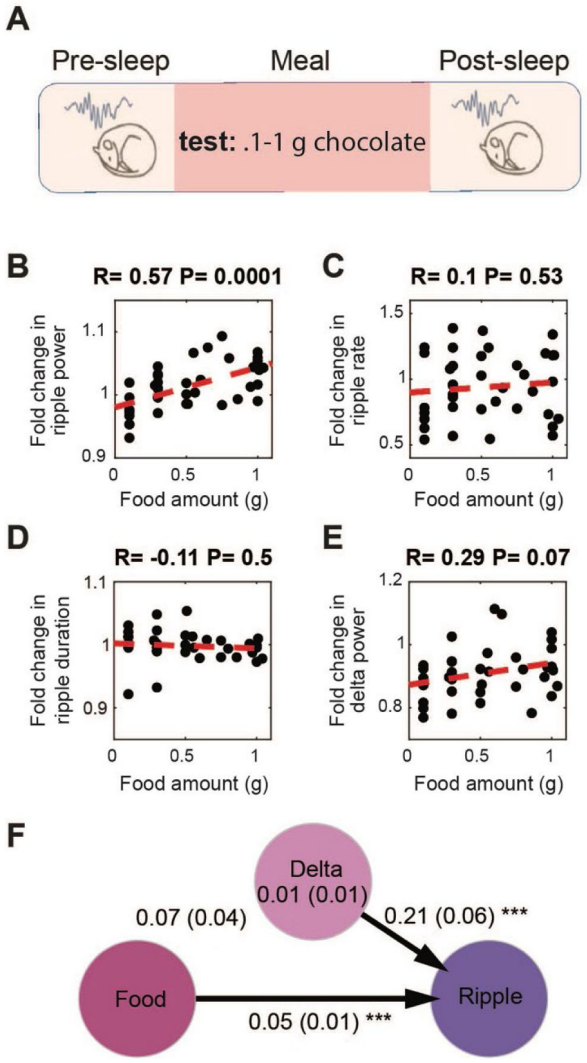

Figure 2.

Food intake enhances SWRs under ad libitum conditions.

A. Experimental design. **B.** Correlation between the amount of chocolate consumed and the degree of the increase in ripple power under ad libitum conditions (N_{animal} = 9, $N_{session}$ = 39, R = .57, p = .0001). **C-E.** correlations between amount of chocolate consumed and fold change in ripple rate (C, R = 0.1, p = 0.53), ripple duration (D, R = -0.11, p = 0.1) and delta power (E, R = 0.29, p = 0.07). **F.** A mediation analysis showed that the effect of chocolate intake on ripple power was significant after accounting for sleep-dependent effects (the indirect effect of the change in delta power mediating the relationship between meal size and ripple power: B = .01, SE = .01, p > .05, the direct effect of meal size on the change in ripple power: B = .05, SE = .01, p < .001).

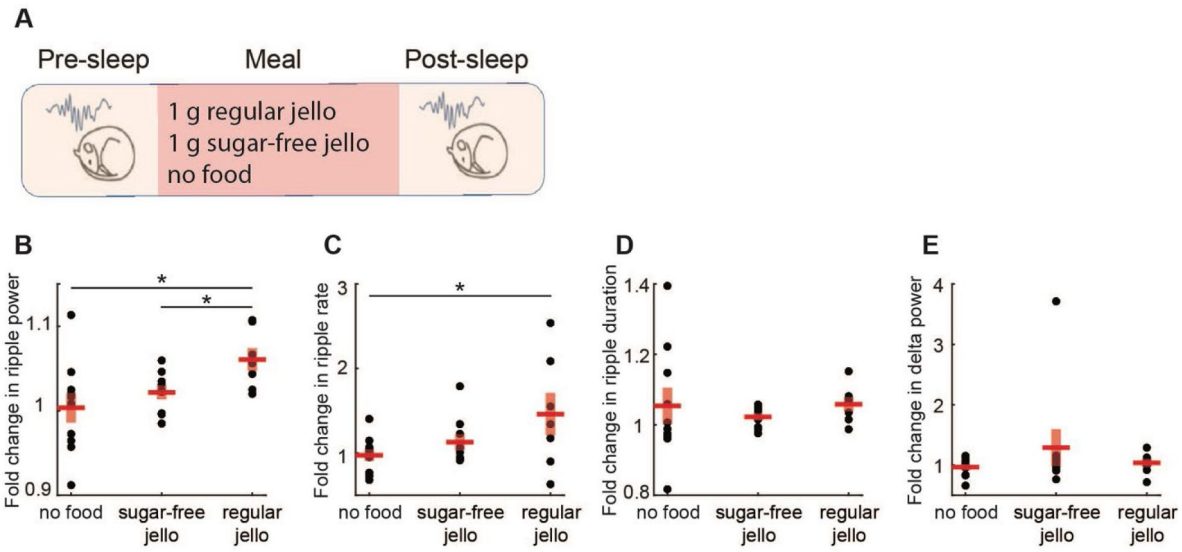

Figure 3.

Caloric content of meals, rather than the experience of food intake, drives the enhancement of SWRs.

A. Experimental design ($N_{regular}$ = 7, $N_{sugar\text{-}free}$ = 9, N_{nofood} = 10). **B**. Consumption of regular Jello resulted in a greater increase in ripple power compared to non-caloric Jello ($t(14) = 2.59$, $p = 0.02$) and the control condition ($t(15) = 2.39$, $p = 0.03$), with no significant difference between sugar-free Jello and the control condition ($p = .38$). **C.** Regular Jello consumption increased SWR rate compared to the control condition ($t(15) = 2.18$, $p = 0.046$), with no other significant differences across conditions (ps > 0.05). No differences were detected in the SWR duration or delta power across conditions (**D-E,** ps > .05). Red lines indicate mean, and shaded red areas indicate standard error. Datapoints within each panel represent different animals.

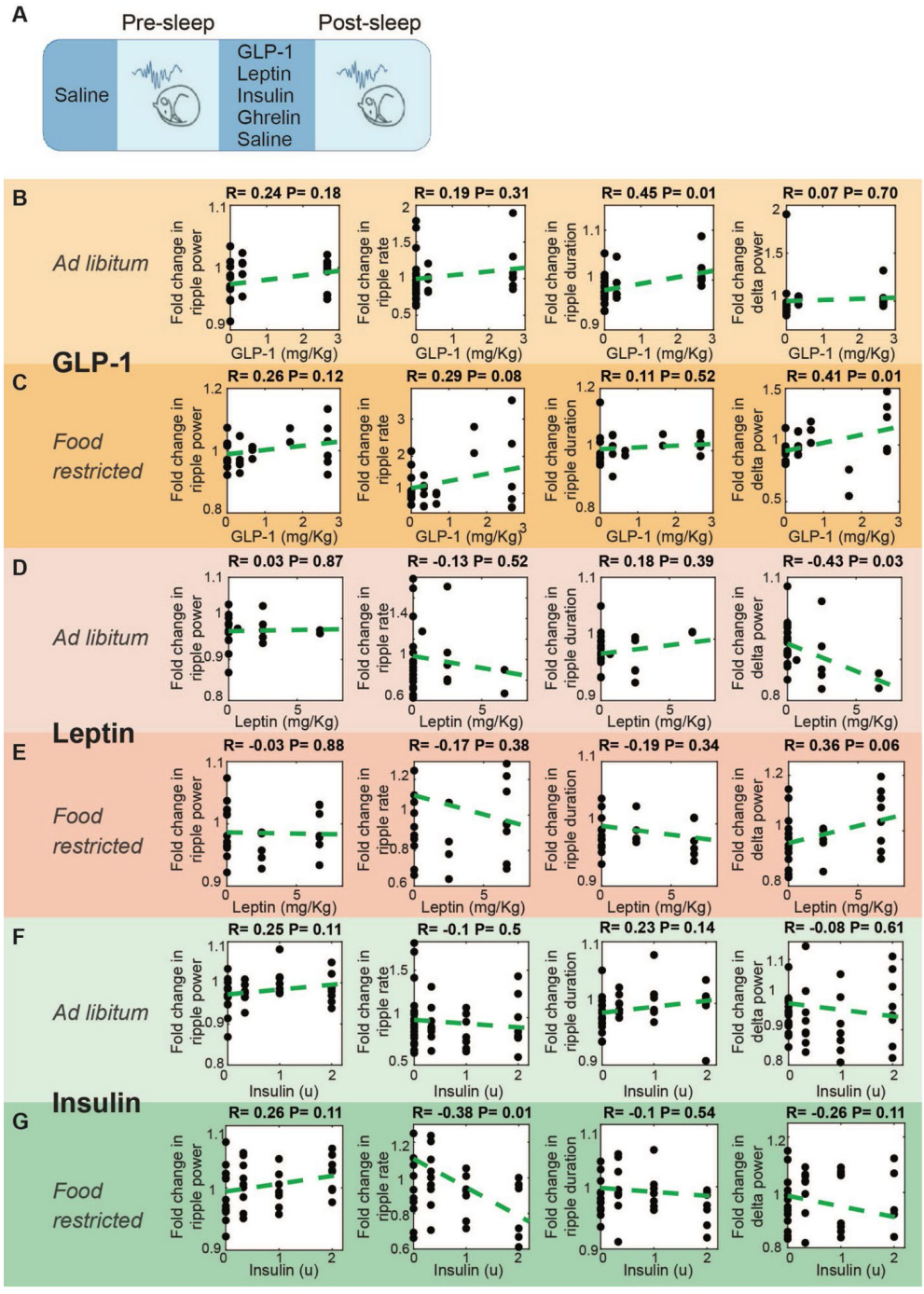

Figure 4.

Effect of systemic administration of hormones on SWR properties.

(A) Experimental design. No dose-dependent effects were detected in ripple power, rate, or duration following the administration of GLP-1 **(B-C)**, leptin **(D-E),** or insulin (**F**) (ps > .05), except a decrease in ripple rate following insulin administration under food-restricted conditions (**G** R = - 0.38, p = 0.01). Ghrelin administration caused a dose-dependent decreased in SWR rate (R = - 0.49, p = 0.0008) and delta power (R = -0.42, p = 0.005) under ad libitum conditions (**H**), and decreased ripple power (R = -0.64, p = 0.0002), ripple rate (R = -0.63, p = 0.0003), and delta power (R = -0.73, p = 0.00014) under food-restricted conditions (**I**). Mediation analysis revealed that ghrelin's effect on ripple power remained significant after accounting for changes in cortical delta power under food-restricted conditions (**I.i, I.ii,** direct effect of ghrelin on ripple power: B = - 0.25, SE = 0.11, p < 0.05).

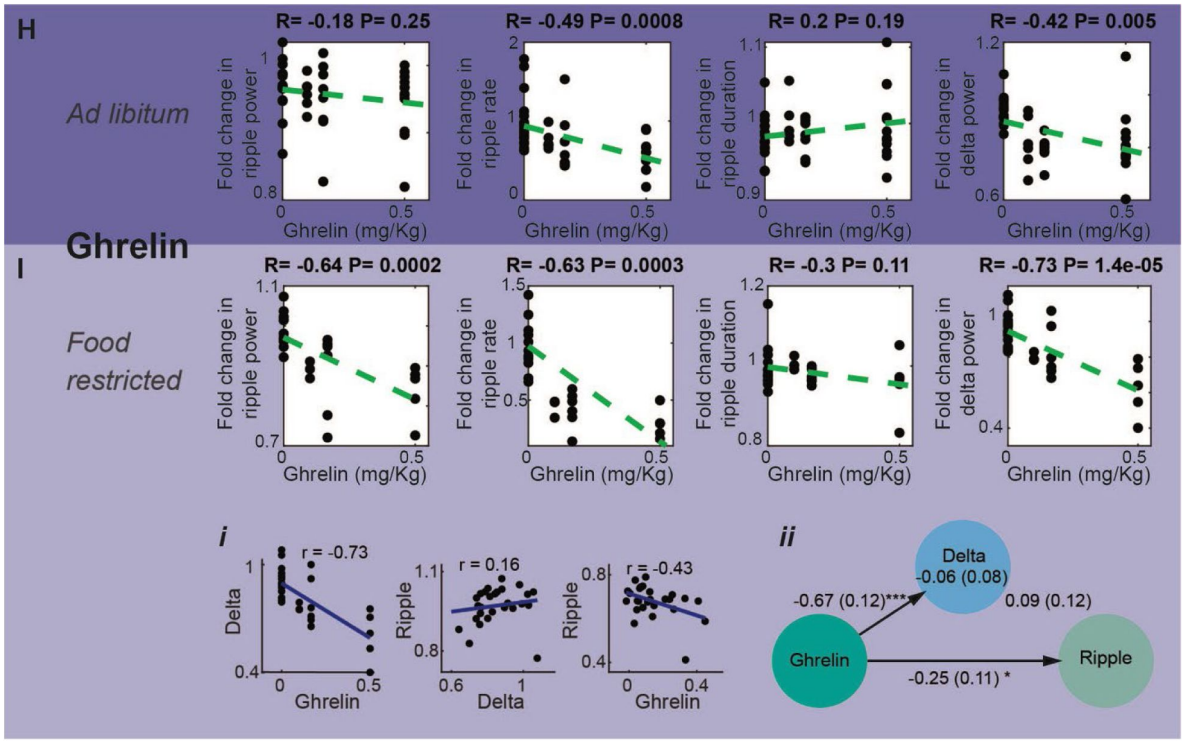

Figure 4. (continued)

jGCaMP8f-WPRE vector (**Fig. 5A-B**). We found that LH GABAergic neurons exhibit a robust and consistent increase in activity, peaking approximately 320 ms after SWR onset (**Fig. 5C-J**, **Suppl. Fig. 2)**. These findings strongly suggest that SWRs, which we found to be modulated by feeding, are also associated with a consistent and robust increase in population activity in the LH, a central brain region for metabolic regulation.

Discussion

Regulation of energy intake and expenditure is vital for animals. While food intake provides essential fuel for the organism, it can also elevate plasma glucose and other nutrient levels above optimal ranges for physiological functions. This elevation triggers coordinated neural and peripheral responses that lower glucose levels by promoting glucose uptake in peripheral tissues and inhibiting further food intake (Chen et al., 2015; Lopez-Gambero et al., 2019; Steculorum et al., 2016; Woods et al., 2006). Here, we show that hippocampal SWRs, previously linked to reductions in peripheral glucose levels (Tingley et al., 2021), are enhanced after food intake. Specifically, we compared SWRs occurring during sleep before and after meals and found a calorie-dependent increase in the ripple power and SWR rate. This enhancement exceeded the increase in sleep quality and was evident under both *ad libitum* and food-restricted conditions. Our caloric vs. non-caloric Jello experiments further revealed that the enhancement in SWRs was driven by caloric intake rather than the experience of eating. Moreover, we found that GABAergic neurons in the lateral hypothalamus, a region implicated in the regulation of both food intake and peripheral metabolism (Anand & Brobeck, 1951; Lopez-Gambero et al., 2019; Shimazu, 1987), exhibit a robust and consistent SWR-triggered increase in activity.

We conducted our experiments during the light phase and focused our analyses on sleep because awake SWRs primarily occur during *offline* states (i.e., resting) (Buzsaki, 2015), which are reduced under food restriction due to increasing physical activity (Chowdhury et al., 2015). However, a bidirectional relationship between hunger and sleep also exists (Danguir & Nicolaidis, 1979; Rechtschaffen & Bergmann, 1995), where hunger increases arousal and impairs sleep quality (Danguir & Nicolaidis, 1979; Goldstein et al., 2018). In line with this, we found that cortical delta power was enhanced following meals in previously food-restricted mice. Nonetheless, mediation analyses showed that food intake still had a significant effect on ripple power, even after accounting for the restoration of sleep quality. To further exclude the possibility that our results were a byproduct of impaired sleep caused by prior food restriction, we performed additional experiments under *ad libitum* food access. Although we did not observe a change in ripple rate in these experiments, we found a dose-dependent increase in ripple power following chocolate consumption, replicating our earlier findings under food restricted conditions. Notably, the extent of SWR modulation was weaker under *ad libitum* conditions, similar to the asymmetric hypothalamic response to food in sated versus fasted animals (Chen et al., 2015).

Conversely, a poor night's sleep leads to increased appetite and impaired metabolic regulation the subsequent day (Hogenkamp et al., 2013; Klingenberg et al., 2013; Schmid et al., 2015). Chronic sleep disturbances, on the other hand, elevate the risk of long-term conditions such as diabetes and metabolic syndrome (Knutson et al., 2007). Although the neural mechanisms underlying these processes remain largely unknown, a recent study linked stronger coupling of sleep spindles and cortical slow-wave activity to improved glucose metabolism regulation the subsequent day in humans (Vallat et al., 2023). Given that about 75% of SWRs occur during NREM sleep in rats (Tingley et al., 2021), and that SWRs are often triggered when slow waves and spindles are coupled (Sirota et al., 2003; Staresina et al., 2015), this suggests that SWRs can play a role in the connection between sleep and next-day metabolic regulation.

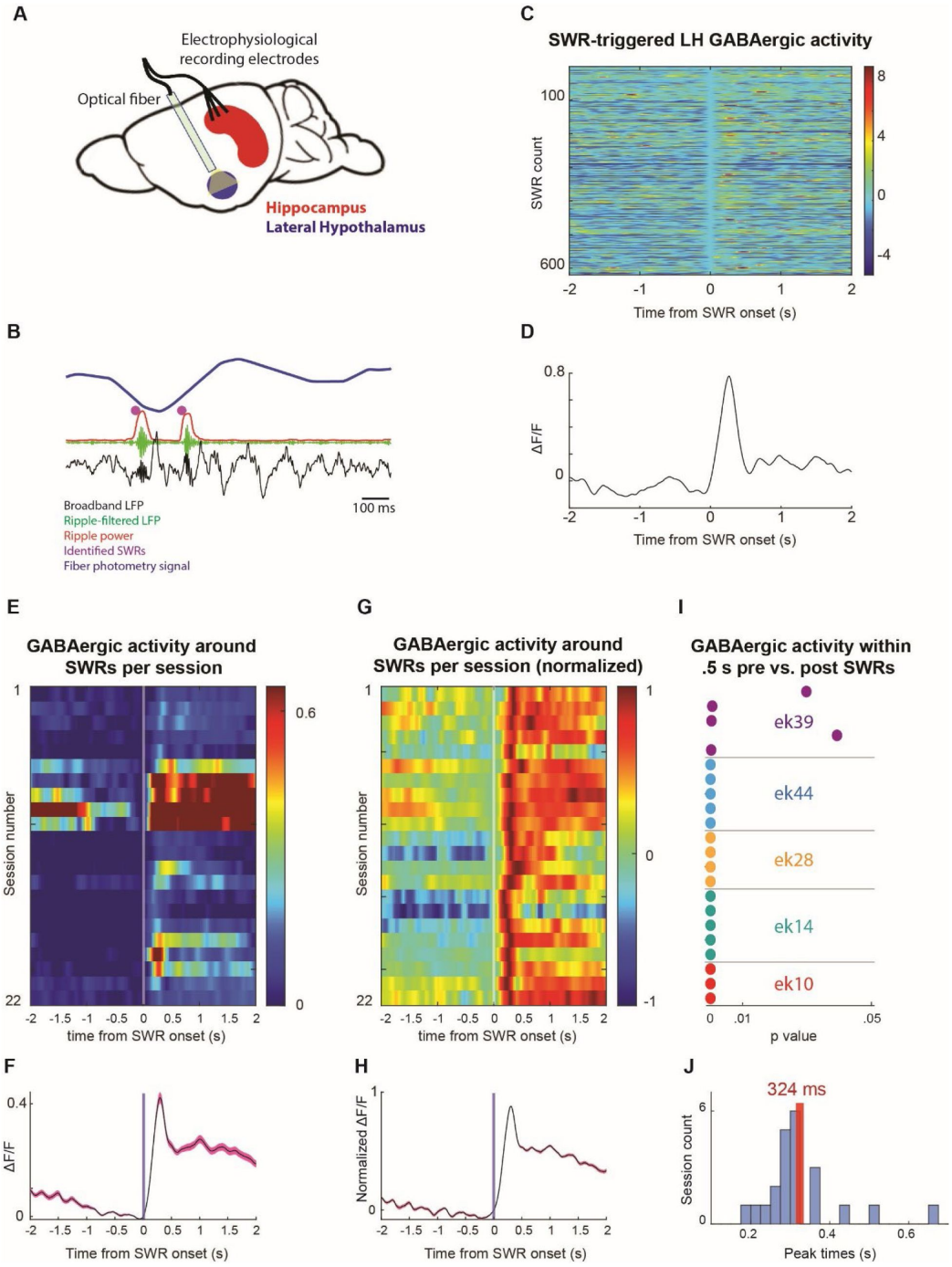

Figure 5.

Lateral hypothalamic GABAergic neuronal populations exhibit an increase in activity following SWRs.

A. Experimental design. **B**. A representative recording showing broadband hippocampal LFP (black), ripple-filtered LFP (150-250 Hz, green), ripple power (red), detected SWRs (purple dots), and the fiber photometry signal from LH GABAergic neurons (FP, blue). **C.** Example traces of LH GABAergic neuronal activity around SWRs. **D**. Mean trace of peri-SWR LH GABAergic activity from one session. **E**. Mean SWR-triggered LH GABAergic activity profiles per session. **F**. The mean of the SWR-triggered LH GABAergic activity profiles across sessions. **G-H**. Similar to E-F but for normalized traces. **I.** Statistical significance of LH GABAergic activity within 0.5 s before and after SWRs per session. **J.** Peak time of LH GABAergic activity relative to SWR onset. Shaded error bars (pink) indicate standard deviation.

The hippocampus, which integrates environmental and interoceptive signals along with past experiences to support spatial navigation and memory, has also been shown to influence food intake (Kanoski & Grill, 2017; Stevenson & Francis, 2017). Notably, HM, a famous patient who lost the ability to form new memories after the removal of his hippocampi, also exhibited irregular eating habits and difficulties with interoceptive judgements (Hebben et al., 1985). Earlier rodent studies similarly showed that hippocampal lesions increase meal frequency in rats (Clifton et al., 1998). More recently, optogenetic inhibition of the hippocampus following meals was shown to decrease the latency to the next meal and increase its size (Hannapel et al., 2019). These findings suggest that hippocampal-dependent memories of recent meals may suppress appetite.

Another study identified dopamine-2 receptor neurons in the dorsal CA3 region of the hippocampus as being responsive to meals and food cues, with inhibition and activation of these neurons increasing and decreasing food intake, respectively (Azevedo et al., 2019). Additional research has highlighted the role of the hippocampus in utilizing peripheral signals related to energy balance for meal regulation (Davidson & Jarrard, 1993; Kanoski & Grill, 2017; Wee et al., 2024). For example, the administration of leptin into both the ventral and dorsal hippocampus (Kanoski et al., 2011), and exendin-4 (a GLP-1 agonist) into the ventral hippocampus (Hsu et al., 2015), have been shown to reduce feeding. Conversely, ghrelin signaling in the ventral hippocampus has been shown to increase meal size through a descending lateral hypothalamus-hindbrain circuit (Suarez et al., 2020). A recent study found distinct neural populations in the dorsal hippocampus that show increased activity following the intake of either fats or sugars, and these populations were involved in memory for the location of the respective foods, as well as in food intake (Yang et al., 2025).

While previous studies employing pharmacological and viral approaches have revealed the role of the hippocampus in the regulation of food intake, understanding at the physiological level has remained limited. Peripheral hormones that rise during the postprandial period, such as insulin, amylin and GLP-1, not only reduce plasma glucose levels but also act as satiety signals that inhibit food intake (Woods et al., 2006). Given this dual inhibitory effect of postprandial signals on both glucose levels and food intake, we propose that SWRs could also play an inhibitory role in regulating food intake. Future studies could explore this possibility by using closed-loop suppression of SWRs (Girardeau et al., 2009). This approach could also be used to test whether SWRs underlie the link between sleep loss and subsequent impairment in glucose metabolism and increase in appetite (Hogenkamp et al., 2013; Schmid et al., 2015; Klingenberg et al., 2013). While we did not find consistent modulation of SWRs following GLP-1, insulin, or leptin administration, we believe this is likely due to specific aspects of our experimental design, such as focusing on the 2-hour Post-sleep period, administering only one hormone at a time, or the use of subcutaneous delivery of the hormones.

While our focus was on metabolic factors affecting SWRs, our results may also have implications for the interaction between memory and metabolism. It is widely recognized that memory tasks guided by food reward enhance SWRs (Ambrose et al., 2016; Singer & Frank, 2009). This modulation, thought to support the consolidation of memory for the correct trajectory leading to food, could also be influenced by food intake and may modulate feeding-related processes. Our Jello experiments showed that the enhancement in SWRs following food intake is dependent on caloric content of meals rather than the experience of eating. However, one might argue that only salient experiences (i.e. consumption of food with calories, but not without) are consolidated, and thus the enhancement of SWRs following regular Jello consumption could reflect the memory consolidation of a salient experience. Previous studies have shown that animals can learn the nutrition content of different foods (Yamamoto & Ueji, 2011). Since our mice had been habituated to all foods before the experiments (regular Jello, sugar-free Jello and chocolate), they might have anticipated that sugar-free Jello would not provide calories (i.e., a less salient experience) or raise glucose levels. However, the enhancement of SWRs for memory consolidation

typically requires novel events. As our mice were well-habituated to both the experimental paradigm and the Jello, it is unlikely that the enhancement in SWRs reflects a memory consolidation process. One potential experiment to bypass mnemonic processes could involve direct gastric infusion of food. Further studies could also investigate whether food consumption after specific events enhances consolidation through the modulation of SWRs.

We found that the SWR property that was most strongly modulated by food is ripple power, followed by SWR rates, while SWR durations were unmodulated by feeding. This is interesting in light of prior studies showing that memory consolidation is linked to an increased rate (Eschenko et al., 2008) and duration (Fernandez-Ruiz et al., 2019) of SWRs or replay events (though reactivation strength also increases(Giri et al., 2019)). Ripple power is strongly correlated with the degree of synchronized recruitment of hippocampal populations (Csicsvari et al., 1999; Schomburg et al., 2012). In contrast, increased SWR rates following learning allow repeated reactivation of recent experience (Eschenko et al., 2008; Giri et al., 2019), while long-duration SWRs have been proposed to facilitate replay of particularly long spatial trajectories (Fernandez-Ruiz et al., 2019). In alignment with the previous finding that replay content is not communicated to subcortical regions involved in metabolic regulation (Tingley & Buzsaki, 2020), ripple power may be the key attribute relevant for homeostatic signaling, while replay content, manifesting in increased SWR rate and duration, underlie the mnemonic function of SWRs.

We propose that the temporal coordination between the onset of SWRs and the increase in LH population activity may represent a mechanism by which SWRs influence peripheral metabolism and food intake. GABAergic lateral septal neurons can facilitate the relay of SWRs to the LH by providing windows of disinhibition (Risold & Swanson, 1997; Tingley et al., 2021; Wong et al., 2016). While our fiber-photometry recordings from the LH provided a population-level activity signal, future studies could utilize single-cell resolution imaging methods to uncover network-level organization and dynamics (Aharoni et al., 2019; Lee & Rothschild, 2021; Vivaldo et al., 2023; Vogt, 2022). Furthermore, while our investigation of hippocampal-LH communication was limited to the timescale of seconds around SWRs, whether and how this communication changes across the timescale of hours following feeding remains to be determined. Finally, the causal relationship between SWRs and LH activity, as well as the functional significance of these interactions, remain to be investigated.

In summary, our study demonstrates that SWRs are sensitive to the body's hunger state and are modulated by food intake. Whether SWRs can, in turn, influence food intake and the specific mechanisms underlying this potential modulation remain to be elucidated.

Methods

Animals

All experimental procedures followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Michigan's Institutional Animal Care and Use Committee. We used C57BL/6 wild-type mice (n = 8, 7 male and one female) and Vgat-IRES-Cre (n = 9 male mice; The Jackson laboratory, #016962) heterozygous mice bred in-house. The mice were housed under a 12:12 light-dark cycle.

Surgeries

Mice (7 to 16 weeks old) were anesthetized with a ketamine-xylazine mixture (100 and 10 mg/kg, respectively; administered via intraperitoneal injection, IP) and received lidocaine and carprofen (4 mg kg-1 and 5 mg kg-1, respectively) prior to being placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.). During the surgery, the mice were kept under isoflurane anesthesia (~1% in O2).

For hippocampal electrophysiology recordings, an array of four tungsten wires (50 micron, California Fine Wire Company) were implanted in the CA1 region of the dorsal hippocampus (AP: -2 mm, ML: 1.5 mm, DV: -1.4 mm). Additionally, an EMG wire was inserted into the neck muscle and an EEG and a ground screw were placed above the parietal cortex and the cerebellum, respectively.

Fiber photometry procedures were done as described previously (Eban-Rothschild et al., 2020; Sotelo et al., 2024). Briefly, 400 nl of pGP-AAV-syn-FLEX-jGCaMP8f-WPRE (Addgene) ($2.6e^{12}$ vg/mL) were injected into the LH (AP: -1.2 mm, ML: 1.15 mm, DV: -5 mm) and mono fiber optic probes (400 µm diameter, 0.48 NA, Doric Lenses, Inc., Quebec, Canada) were implanted just above the LH (AP: -1.5 mm, ML: 1.15 mm, DV: - 4.6 mm).

Mice were given several days to recover from surgery. They were then habituated to handing and individually housed in custom Plexiglas recording cages (39.4 cm x 28.6 cm × 19.3 cm) with open tops to ensure that the LFP cable could reach the mice and allow them to be freely behaving without restricted movements. We recorded mice until 1 year of age.

Data processing, analyses and sleep scoring

Electrophysiological and optical recordings were conducted using the Tucker Davis Technology (TDT) system, sampled at 3 kHz and 1kHz respectively. Data was visualized with Synapse or SynapseLite software. Sleep scoring was done manually using the AccuSleep toolbox (*https://github.com/zekebarger/AccuSleep*) using the EEG and EMG signals. NREM periods were detected based on high EEG delta power and low EMG power, REM periods were detected based on high EEG theta power and low EMG power, and Wake periods were detected based on high EMG power. Only NREM periods were included for subsequent SWR detection, quantification and analyses (in particular, reported SWR rates reflect the number of SWRs per second of NREM sleep). Sessions with less than 30 minutes of sleep were excluded from further analyses.

To detect SWRs we filtered the raw signal between 150-250 Hz, and took an envelope of the squared filtered signal. We identified SWRs by identifying the events that reached at least 5 standard deviations above the mean and that were above 1 standard deviation for at least 40 ms. In case two events occurred within 40 ms or less, we discarded the second event. Ripple power threshold calculations were done together for Pre-sleep and Post-sleep. We calculated the delta power by taking the envelope of the squared filtered signal between 0.5-4 Hz on the EEG channel during NREM sleep.

For Pre-sleep and Post-sleep comparisons, we calculated average ripple power, ripple rate, ripple duration and delta power for each pre and post session. Change from Pre-sleep to Post-sleep was calculated as change=mean($\text{variable}_{\text{postsleep}}$)/mean($\text{variable}_{\text{presleep}}$). Mediation analyses were done using the Mediation toolbox (*https://github.com/canlab/MediationToolbox*).

For SWR-LH interaction analyses, we interpolated the fiber photometry signal to match its length to the length of the LFP. For each SWR, we extracted the surrounding 4-second windows of the fiber photometry signal and centered the fiber photometry signal at the ripple onset to zero. We detected the SWR-triggered peaks in fiber photometry signal within the 1 sece following SWRs. For statistical comparisons, we calculated the integrals of the 0.5 sec windows before and after each SWR and performed a paired t-test on these integrals. For the summary plots, we normalized each session by its own maximum value.

Food restriction and experimental procedures

All experiments were conducted during the light phase. Chow experiments were conducted under food restricted conditions in which animals did not have access to food for 20-24 hours preceding the experiments. Experimental steps were as follows: Mice were left to sleep for 2 hours (Pre-

sleep). Then they were woken up and given a piece of regular chow (Meal). The amount of chow varied between 0.3-1.5 grams. To calculate the exact amount of consumption, we removed and weighed any remaining chow pieces before the start of the Post-sleep session which lasted for another 2 hours. Each gram of chow contained 4.1 kcals. For chocolate experiments, mice went through the same experimental procedure except that they had *ad libitum* food access throughout the experiment and received varying amounts (0.1-1 gr) of Reese's Peanut Butter Cups instead of chow. Each gram of chocolate contained 5 kcals.

Jello experiments were performed under food-restricted conditions by using regular or sugar-free strawberry flavored Jello. Each 10 gr of Jell-O gelatin mix was dissolved in 13 ml water for regular Jello meals, and each 10 gr of sugar-free strawberry flavored Jell-o gelatin mix was dissolved in 111 ml water for sugar-free meals. The mixtures were refrigerated (4 Celsius) until firm. One gr of regular and sugar-free Jello meals contained 1.7 and 0 kcals, respectively. The sugar free Jello contained artificial sweeteners (Aspartame and Acesulfame Potassium). The Jello meals were given on plastic weighing boats and weighed before and after the trial to calculate the exact amount of consumption.

Hormone experiments

The experiments were conducted under 24-hour food restriction or *ad libitum* food access conditions. No food pieces were present in the cages during the recordings. Before Pre-sleep sessions, mice received a subcutaneous injection of 0.1 ml saline. Before Post-sleep sessions, they were administered with either 0.1 ml saline or various doses of hormones, including ghrelin (0.1, 0.167, or 0.5 mg/kg body weight, Genscript), leptin (0.3, 3, 6.67 mg/kg, Peprotech), insulin (0.5, 1 or 2 u/kg body weight, Sigma Aldrich, I5500), or GLP-1 (0.33, 0.67, 1.67 or 2.67 mg/kg body weight, Tocris, Cat. No. 2082), all dissolved in 0.1 ml saline.

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Additional information

Author contributions

E.K. conceived the experiments with contributions from G.R. and A.E-R.. E.K., E.W., A.A.D. and H.D. conducted the experiments. E.K. and G.R. analyzed data. E.K wrote the manuscript with contributions from G.R. and A.E-R

Additional files

Supplementary figures

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Reviewer #1 (Public review):

Summary:

This manuscript by Kaya et al. studies the effect of food consumption on hippocampal sharp wave ripples (SWRs) in mice. The authors use multiple foods and forms of food delivery to show that the frequency and power of SWRs increase following food intake, and that this effect depends on the caloric content of food. The authors also studied the effects of administration of various food-intake-related hormones on SWRs during sleep, demonstrating that ghrelin negatively affects SWR rate and power, but not GLP-1, insulin, or leptin. Finally, the authors use fiber photometry to show that GABAergic neurons in the lateral hypothalamus, increase activity during a SWR event.

Strengths:

The experiments in this study seem to be well performed, and the data are well presented, visually. The data support the main conclusions of the manuscript that food intake enhances hippocampal SWRs. Taken together, this study is likely to be impactful to the study of the impact of feeding on sleep behavior, as well as the phenomena of hippocampal SWRs in metabolism.

Weaknesses:

None

https://doi.org/10.7554/eLife.105059.2.sa3

Reviewer #2 (Public review):

Summary:

Kaya et al uncover an intriguing relationship between hippocampal sharp wave-ripple production and peripheral hormone exposure, food intake, and lateral hypothalamic function. These findings significantly expand our understanding of hippocampal function beyond mnemonic processes and point a direction for promising future research.

Strengths:

Some of the relationships observed in this paper are highly significant. In particular, the inverse relationship between GLP1/Leptin and Insulin/Ghrelin are particularly compelling as this aligns well with opposing hormone functions on satiety.

https://doi.org/10.7554/eLife.105059.2.sa2

Reviewer #3 (Public review):

Summary:

The manuscript by Kaya et al. explores the effects of feeding on sharp wave-ripples (SWRs) in the hippocampus, which could reveal a better understanding of how metabolism is regulated by neural processes. Expanding on prior work that showed that SWRs trigger a decrease in peripheral glucose levels, the authors further tested the relationship between SWRs and meal consumption by recording LFPs from the dorsal CA1 region of the hippocampus before and after meal consumption. They found an increase in SWR magnitude during sleep after food intake, in both food-restricted and ad libitum fed conditions. Using fiber photometry to detect GABAergic neuron activity in the lateral hypothalamus, they found increased activity locked to the onset of SWRs. They conclude that the animal's satiety state modulates the amplitude and rate of SWRs, and that SWRs modulate downstream circuits involved in regulating feeding.

The authors have addressed prior requests for revision and clarification, and provide a convincing case for SWRs being modulated by satiety state. These experiments provide an important step forward in understanding how metabolism is regulated in the brain. The study will likely be of great interest in the field of learning and memory while carrying broader implications for understanding brain-body physiology.

https://doi.org/10.7554/eLife.105059.2.sa1

Author response:

The following is the authors' response to the original reviews

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *This manuscript by Kaya et al. studies the effect of food consumption on hippocampal sharp wave ripples (SWRs) in mice. The authors use multiple foods and forms of food delivery to show that the frequency and power of SWRs increase following food intake, and that this effect depends on the caloric content of food. The authors also studied the effects of the administration of various food-intake-related hormones on SWRs during sleep, demonstrating that ghrelin negatively affects SWR rate and power, but not GLP1, insulin, or leptin. Finally, the authors use fiber photometry to show that GABAergic neurons in the lateral hypothalamus, increase activity during a SWR event.*
>
> *Strengths:*
>
> *The experiments in this study seem to be well performed, and the data are well presented, visually. The data support the main conclusions of the manuscript that food intake enhances hippocampal SWRs. Taken together, this study is likely to be impactful to the study of the impact of feeding on sleep behavior, as well as the phenomena of hippocampal SWRs in metabolism.*
>
> *Weaknesses:*
>
> *Details of experiments are missing in the text and figure legends. Additionally, the writing of the manuscript could be improved.*

We thank the reviewer for their favorable assessment of the work and its potential impact. We have added all requested details in the text and figure legends and revised the wording of the manuscript to improve its clarity.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *Kaya et al uncover an intriguing relationship between hippocampal sharp wave-ripple production and peripheral hormone exposure, food intake, and lateral hypothalamic function. These findings significantly expand our understanding of hippocampal function beyond mnemonic processes and point a direction for promising future research.*
>
> *Strengths:*
>
> *Some of the relationships observed in this paper are highly significant. In particular, the inverse relationship between GLP1/Leptin and Insulin/Ghrelin are particularly compelling as this aligns well with opposing hormone functions on satiety.*
>
> *Weaknesses:*
>
> *I would be curious if there were any measurable behavioral differences that occur with different hormone manipulations.*

We thank the reviewer for their favorable assessment of the work and its contribution to our understanding of non-mnemonic hippocampal function. Whether there are behavioral differences that occur following administration of the different hormones is a great question, yet unfortunately our study design did not include fine behavioral monitoring to the degree that would allow answering it. While some previous studies have partially addressed the behavioral consequences of the delivery of these hormones (and we reference these studies in our Discussion), how these changes may interact with the hippocampal and hypothalamic effects we observe is a very interesting next step.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *The manuscript by Kaya et al. explores the effects of feeding on sharp wave-ripples (SWRs) in the hippocampus, which could reveal a better understanding of how metabolism is regulated by neural processes. Expanding on prior work that showed that SWRs trigger a decrease in peripheral glucose levels, the authors further tested the relationship between SWRs and meal consumption by recording LFPs from the dorsal CA1 region of the hippocampus before and after meal consumption. They found an increase in SWR magnitude during sleep after food intake, in both food restricted and ad libitum fed conditions. Using fiber photometry to detect GABAergic neuron activity in the lateral hypothalamus, they found increased activity locked to the onset of SWRs. They conclude that the animal's satiety state modulates the amplitude and rate of SWRs, and that SWRs modulate downstream circuits involved in regulating feeding. These experiments provide an important step forward in understanding how metabolism is regulated in the brain. However, currently, the paper lacks sufficient analyses to control for factors related to sleep quality and duration; adding these analyses would further support the claim that food intake itself, as opposed to sleep quality, is primarily responsible for changes in SWR activity. Adding this, along with some minor clarifications and edits, would lead to a compelling case for SWRs being modulated by a satiety state. The study will likely be of great interest in the field of learning and memory while carrying broader implications for understanding brain-body physiology.*

> *Strengths:*
>
> *The paper makes an innovative foray into the emerging field of brain-body research, asking how sharp wave-ripples are affected by metabolism and hunger. The authors use a variety of advanced techniques including LFP recordings and fiber photometry to answer this question. Additionally, they perform comprehensive and logical follow-up experiments to the initial food-restricted paradigm to account for deeper sleep following meal times and the difference between consumption of calories versus the experience of eating. These experiments lay the groundwork for future studies in this field, as the authors pose several follow-up questions regarding the role of metabolic hormones and downstream brain regions.*

We thank the reviewer for their appreciation and constructive review of the work.

> *Weaknesses:*
>
> *Major comments:*
>
> *(1) The authors conclude that food intake regulates SWR power during sleep beyond the effect of food intake on sleep quality. Specifically, they made an attempt to control for the confounding effect of delta power on SWRs through a mediation analysis. However, a similar analysis is not presented for SWR rate. Moreover, this does not seem to be a sufficient control. One alternative way to address this confound would be to subsample the sleep data from the ad lib and food restricted conditions (or high calorie and low calorie, etc), to match the delta power in each condition. When periods of similar mean delta power (i.e. similar sleep quality) are matched between datasets, the authors can then determine if a significant effect on SWR amplitude and rate remains in the subsampled data.*

This is an important point that we believe we addressed in a few complementary ways. First, the mediation analysis we implemented measures the magnitude and significance of the contribution of food on SWR power after accounting for the effects of delta power, showing a highly significant food-SWR contribution. While the objective of subsampling is similar, mediation is a more statistically robust approach as it models the relationship between food, SWR power, and delta power in a way that explicitly accounts for the interdependence of these variables. Further, subsampling introduces the risk of losing statistical power by reducing the sample size, due to exclusion of data that might contain relevant and valuable information. Mediation analysis, on the other hand, uses the full dataset and retains statistical power while modeling the relationships between variables more holistically. However, as we were not satisfied with a purely analytical approach to test this issue, we carried out a new set of experiments in ad-libitum fed mice, where there is no concern of food restriction impairing sleep quality in the presleep session. In these conditions food amount also significantly correlated with, and showed significant mediation of, the SWR power change. Finally, we acknowledge and discuss this point in the Discussion, highlighting that given the known relationship between cortical delta and SWRs, it is challenging to fully disentangle these signals.

> *(2) Relatedly, are the animals spending the same amount of time sleeping in the ad lib vs. food restricted conditions? The amount of time spent sleeping could affect the probability of entering certain stages of sleep and thus affect SWR properties. A recent paper (Giri et al., Nature, 2024) demonstrated that sleep deprivation can alter the magnitude and frequency of SWRs. Could the authors quantify sleep quantity and control for the amount of time spent sleeping by subsampling the data, similar to the suggestion above?*

Following the reviewer's comment, we have quantified and compared the amount of time spent in NREM sleep in the Pre and Post session pairs in which the animals were food restricted, with 0-1.5 g of chow given between the sleep sessions. We found that there was no significant difference in the amount of time spent in NREM sleep in the Pre and Post sessions. We have added this result to the Results section of the manuscript and as a new Supplementary Fig. 1.

Additionally, we have added details to the Methods section that were missing in the original submission that are relevant to this point. Specifically, within the sleep sessions, the ongoing sleep states were scored using the AccuSleep toolbox (https://github.com/zekebarger/AccuSleep) using the EEG and EMG signals. NREM periods were detected based on high EEG delta power and low EMG power, REM periods were detected based on high EEG theta power and low EMG power, and Wake periods were detected based on high EMG power. Importantly, only NREM periods were included for subsequent SWR detection, quantification and analyses (in particular, reported SWR rates reflect the number of SWRs per second of NREM sleep).

> *(3) Plot 5I only reports significance but does not clearly show the underlying quantification of LH GABAergic activity. Upon reading the methods for how this analysis was conducted, it would be informative to see a plot of the pre-SWR and post-SWR integral values used for the paired t-test whose p-values are currently shown. For example, these values could be displayed as individual points overlaid on a pair of boxand-whisker plots of the pre- and post-distribution within the session (perhaps for one example session per mouse with the p-value reported, to supplement a plot of the distribution of p-values across sessions and mice). If these data are non-normal, the authors should also use a non-parametric statistical test.*

We have generated the summary plots the reviewer requested and have now included them in Supplementary Fig. 2.

> *Minor comments:*
>
> *(4) A brief explanation (perhaps in the discussion) of what each change in SWR property (magnitude, rate, duration) could indicate in the context of the hypothesis may be helpful in bridging the fields of metabolism and memory. For example, by describing the hypothesized mechanistic consequence of each change, could the authors speculate on why ripple rate may not increase in all the instances where ripple power increases after feeding? Why do the authors speculate that ripple duration does not increase, given that prior work (Fernandez-Ruiz et al. 2019) has shown that prolonged ripples support enhanced memory?*

This is an interesting point and we have added a section to the Discussion to discuss it (pg. 17, last paragraph)

> *(5) The authors suggest that "SWRs could modulate peripheral metabolism" as a future implication of their work. However, the lack of clear effects from GLP-1, leptin and insulin complicates this interpretation. It might be informative for readers if the authors expanded their discussion of what specific role they speculate that SWRs could play in regulating metabolism, given these negative results.*

We have added a section to the Discussion proposing potential reasons for this point (pg. 16, last paragraph)

> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations for the authors):***
>
> *Major Comments:*
>
> *(1) The experiments involve very precise windows of time for sleeping and eating that seem impossible to control. For example, the authors state that for the experiments in Figure 1, there was a 2-h sleep period, followed by a 1-h feeding period, followed by another 2-h sleep period. Without sleep deprivation procedures or other environmental manipulations, how can these periods be so well-defined? Even during the inactive period, mice typically don't sleep for 2-h bouts at once, and the addition of food would not likely lead to an exact 1-h period of wakefulness in the middle. The validity of these experimental times would be more believable if the authors provided much more data on these sessions. For example, the authors could provide a table or visual display of data for the actual timing of the pre-sleep, eating, and post-sleep phases with exact time measurements and/or visual display of sleep versus wakefulness.*

This is an important point, which we were not clear enough about in the original submission. While the durations of the Pre-sleep, Wake and Post-sleep sessions were indeed 2 h, 1 h and 2 h respectively, the animals did not actually sleep during the entirety of the sleep sessions. Importantly, we performed sleep state scoring on all sessions, and only analyzed identified NREM sleep for all SWR analyses. Following the reviewer's comment (and that of Reviewer 1), we have quantified and compared the amount of time spent in NREM sleep in the Pre and Post session pairs in which the animals were food restricted and 0-1.5 g of chow were given between the sleep sessions. We found that there was no significant difference in the amount of time spent in NREM sleep in the Pre and Post sessions. We have added this result to the Results section of the manuscript and as a new Supplementary Fig. 1.

Additionally, we have added details to the Methods section that were missing in the original submission that are relevant to this point. Specifically, within the sleep sessions, the ongoing sleep states were scored using the AccuSleep toolbox (https://github.com/zekebarger/AccuSleep) using the EEG and EMG signals. NREM periods were detected based on high EEG delta power and low EMG power, REM periods were detected based on high EEG theta power and low EMG power, and Wake periods were detected based on high EMG power. Importantly, only NREM periods were included for subsequent SWR detection, quantification and analyses (in particular, reported SWR rates reflect the number of SWRs per second of NREM sleep).

> *(2) I may have missed this (although I tried searching in the text and figure legend), but the authors did not state the difference between green versus red bar colors in Figure 1 C-E. For Figures 1 F-J, do the individual dots represent both the test (fed) animals and control animals, or just the test animals?*

We thank the reviewer for the opportunity to clarify these points. Red bars in Fig. 1C-E represent the SWR changes observed following delivery of equal or more than 0.5 g of chow, while the green bars represent the changes observed following delivery of less than 0.5 g. Fig. 1F-J includes both the experimental and control animals- the control animals appearing as having received 0 food amount. This information has now been added to the figure legend.

> *(3) For the jello experiments in Figure 3, was there only 1 trial per animal? Previous studies show that animals learn the caloric value of jello after subsequent trials, so whether or not multiple trials took place in each animal is important for interpretation of the results.*

In Figure 3, the datapoints within each panel represent different animals and this information has now been added to the figure legend. Nevertheless, the animals were previously habituated to all foods, including regular jello, sugar-free jello and chocolate. While we consider it unlikely that this prior experience was sufficient to underlie the differential effects on SWRs, we cannot fully rule out the possibility that it provided some ability to predict the caloric value and consequences of the different foods. We have added details to the acknowledgement of this point in the Discussion (pg. 17, second paragraph).

> *(4) The experiments in Figure 5 are informative but don't relate to the experiments in the rest of the study. It is difficult to interpret their meaning given that these experiments take place over seconds while the other experiments take place over hours. Some attempt should be made to bridge these experiments over the timescales relevant for the behaviors studied in Figures 1-4.*

We have now further acknowledged and discussed the point that our investigation is limited to the timescale of seconds around SWRs, and thus identified a potential communication channel, but whether and how this communication changes across hours following feeding remains for future studies (pg. 18, second paragraph).

> *(5) Figure 5B should depict the x-axis in seconds, not an arbitrary set of times from a recording.*

We have replaced these with a time scale bar.

> *Minor Comments:*
>
> *(6) The writing of the manuscript can be improved in many places:*
>
> *Sometimes the writing could be more precise. For example, the Abstract states: "hippocampal sharp wave ripples (SWRs)... have been shown to influence peripheral glucose metabolism." Could this be written in a more informative way, rather than just staying "has been shown to influence?" A few more words would provide a lot more information. Similarly, at the end of the Introduction: "we set out to test the hypothesis that SWRs are modulated following meal times as part of the systems-level response to changing metabolic needs." This is not a strong hypothesis... could it be written to boldly state how the SWRs will be modulated (increase or decrease) and provide more assertive information?*
>
> *The writing can be grandiose at times. Phrases such as "life is a continuous journey" or "the hypothalamus is a master regulator of homeostasis" are a bit sophomoric and too colloquial.*
>
> *Finally, a representative recording should be referred to as just that-a "representative recording," as opposed to a "snippet," which is also colloquial. This word is used in the figure legends to Figures 1 and 5, and misspelled as "sinpper" in Figure 1*

We have reworded all these sentences and phrases to make them clearer, more concrete and more formal.

> *(7) The methods state that the study used both male and female mice. Were they used in equal numbers across experiments?*

Only one female was used in the final dataset, and we have corrected the wording accordingly.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *Great paper!*

Thanks!

> ***Reviewer #3 (Recommendations for the authors):***
>
> *Below are some minor requests for clarification, including in figures:*
>
> *(1) Fig. 5H y-axis should say "normalized dF/F."*

Done

> *(2) Fig. 1B is missing a y-axis label. It may be clearer to display separate y-axis scale bars for each component (SWR envelope, ripple-filtered amplitude, etc).*

Done

> *(3) Please include labels for brain areas and methodological components in Fig. 5A.*

Done

> *(4) Should Fig. 5B have the same y-axis or scale bars as 1B?*

We have edited the figure labels and legends to be visually similar

> *(5) In Fig. 5J, is the y-axis a count of sessions?*

Yes, we have added that to the y-axis label

> *(6) Could the authors please clarify whether the sugar-free jello was sweetened with an artificial sweetener? If so, this is a robust control for the rewarding nature of the two jellos, so a quick clarification would highlight this strength of the experiment.*

We thank the reviewer for this great point. Indeed, the sugar free jello contained artificial sweeteners (Aspartame and Acesulfame Potassium). We have added this information to the Results and Methods.

> *(7) It appears in Fig. 5 that there may be a reliable dip in activity **at** the time of SWR onset, followed by the increase afterward, as shown in the example FP trace and the individual ripple-triggered traces. Is this indeed the case, and does this dip fall significantly below baseline? This characterization would be interesting, but I acknowledge is not necessarily crucial to the study to include.*

This would indeed be an interesting finding, but upon examination and statistical testing, we found that this is not the case. We believe this may appear as such due to the normalization of the traces.

> *(8) The authors mention a reduction in ripple rate following insulin under food restriction as the only significant effect for insulin, GLP-1, and leptin, yet there was also a significant increase (at $p<0.05$) in ripple duration for GLP-1 in the ab lib condition. Is this not considered noteworthy?*

This is a fair point and we have reworded the description of this result to simply state that there were no robust, consistent, dose-dependent effects of GLP-1, leptin and insulin on SWR attributes.

https://doi.org/10.7554/eLife.105059.2.sa0