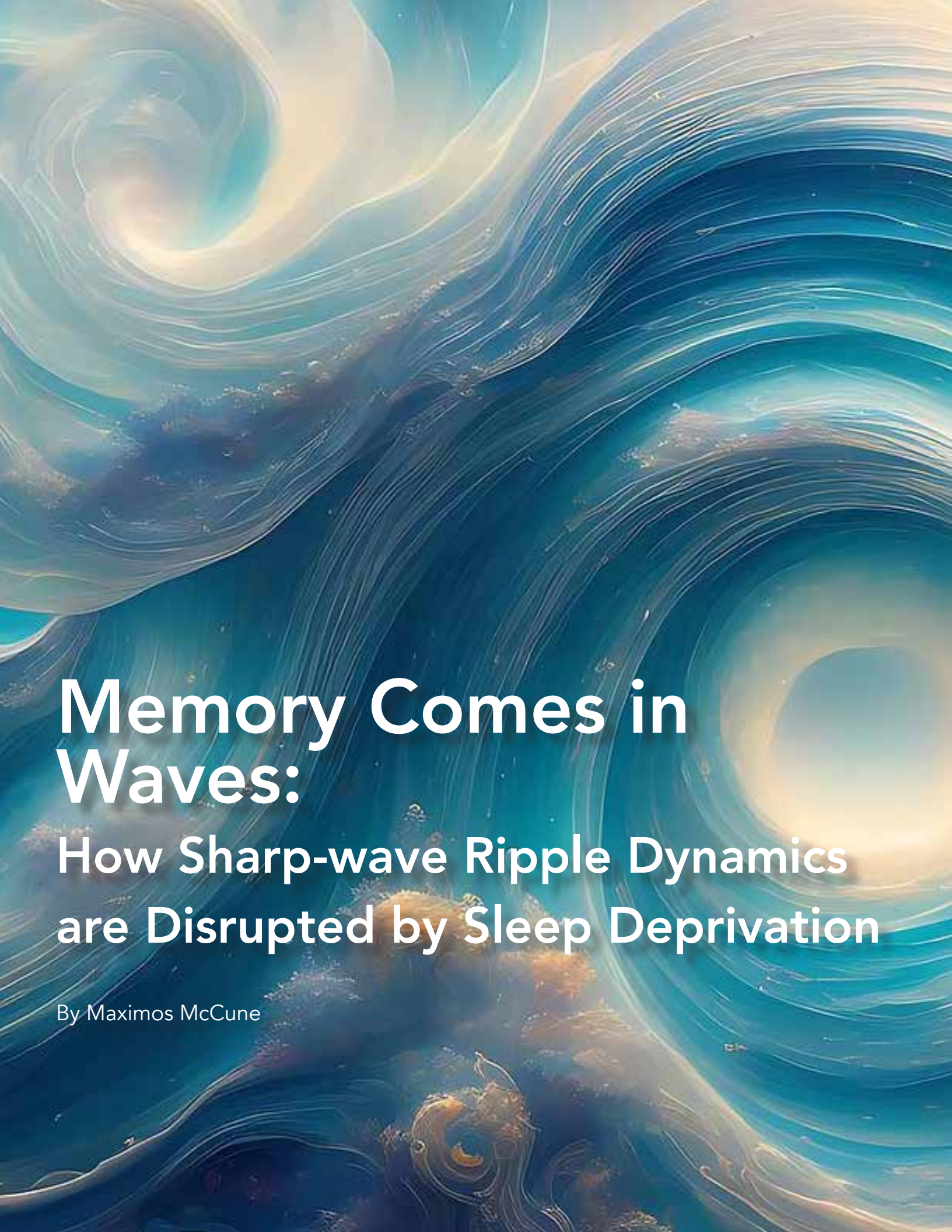


Memory Comes in Waves:

How Sharp-wave Ripple Dynamics are Disrupted by Sleep Deprivation

By Maximos McCune

The feeling of heavy eyelids and slowed breathing is, depending on the day, either something we eagerly welcome or desperately chase away with bright lights and caffeine. On the surface, we recognize sleep as a state of reduced activity, lacking alertness and movement. When we see a friend snoozing on the couch or a stranger taking a catnap on the bus, we typically associate their stillness with them being asleep. Certain parts of the brain, however, act quite differently when you shut your eyes at night. True, some sections of the cortex adopt slower, more rhythmic firing patterns during sleep. But as you may have guessed from your dreams, where running from monsters or flying through the sky are somewhat regular ventures, other brain regions are hard at work while the rest of your body lays still.

Most of us have heard at some point that getting good sleep is important for learning and drives mental performance, but why is this? Is an early bed time really the best course of action before a big exam or job interview? Why not just cram-study for another hour? C'mon, 2 a.m. isn't that late… but is it? A 2009 study from the Journal of Adolescent Health reports that up to a staggering 70% of college students meet the clinical criterion for sleep deprivation [1]. Additionally, a quarter of the 1,125 student survey participants reported at least one sleepless night in the month leading up to the study, with 35% of these students report staying up past 3 a.m. at least once a week [1]. College students are, rather notoriously, plagued with irregular and inadequate sleep, as it is not uncommon to hear a classmate lament the inevitable, procrastination-induced all-nighter. This data highlights the ubiquity of sleep deprivation in student populations, but how does this translate to academic performance? It makes intuitive sense that a week of poor sleep could explain the occasional failed exam or slip-up during a class presentation, but recent works suggest that chronic sleep deprivation is the root cause of something more sinister. In fact, the ramifications of poor sleep extend far beyond short-term mishaps, as scientists have linked sleep deprivation with plummets in grade point average and even decreases in the likelihood of completing school and obtaining a college degree [2]. In other words, sleep is a strong predictor of academic success.

Taking this a step further, a recent investigation aimed to determine the effects of sleep loss on reaction time and cognitive processing speed in adolescents. The study induced 24 hours of sleep deprivation in healthy participants, ages 14-16. To test the effect of one sleepless night on mental performance, the researchers subjected sleepy participants to a memory and reaction time game, in which they were assessed on their ability to press a button upon the presentation of specific visual stimuli. After only one day of sleep deprivation, participants exhibited harsh deficits in psychomotor processing, manifesting as an increase in the number of incorrect button presses and a decrease in reaction time [3]. This sudden drop in performance is rather troublesome—especially considering that these individuals were in good health, well slept prior to the study, and deprived of only a single night's rest. Imagine how these findings would scale in chronically sleep-deprived individuals that work under high-stress or in cognitively demanding environments.

As spooky as this is, it wasn't until recently that scientists and physicians began to piece together the neurobiological story of sleep. Advances in neuroimaging tools and polysomnography have since allowed for researchers to take a closer look at the biological mechanisms that govern sleep [4], suggesting novel answers to the long-puzzling enigma: the connection between sleep and memory [3],[5],[6]. According to the current literature, proper sleep is necessary for long-term memory consolidation and modification [1],[6],[7],[8],[9]. Those mornings that whir past like some fuzzy mirage, calling a long-time colleague or loved one by the wrong name, or that hazy, mid-afternoon brain fog are all too familiar circumstances for most of us. So, pour out your Starbucks and shut off the lights; your brain will thank you in the morning.

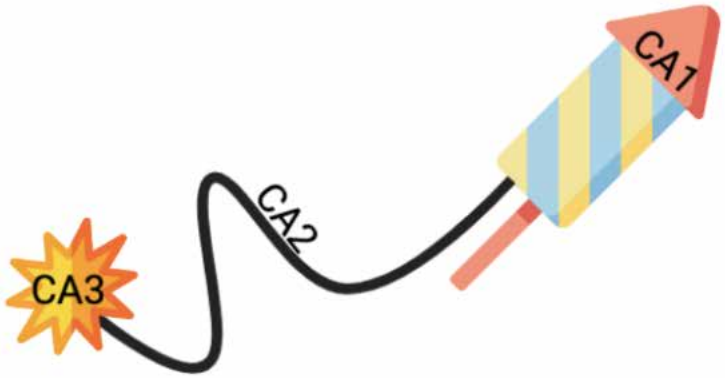


Figure 1*: The firing pattern of hippocampal layers. Created in Biorender; designed using images from Flaticon.com: Hippocampal Firework by Maximos P. McCune under CC BY 4.0.*

It's Getting Late, and Pavlov's Dog has Work in the Morning

We now understand that missing sleep can have disastrous effects on our ability to process new information and form memories, but how do we test this? Well—ethically—of course, but is there a way to accurately assess what causes sleep loss to be so detrimental without causing undue harm? Using human test subjects for studying chronic sleep deprivation is obviously off the table, which leaves us searching for alternatives. If we can't study sleep deprivation in humans, what about an animal model? Well, the use of animals for the study of learning, memory and sensory processing presents more questions than answers. How do the brains of lab rats even match up with their human analogues? Is there even a way to reliably determine whether a non-human animal has learned a process or established a new memory? As you might imagine, studying the conceptual marvel that is human memory has proved to be a rather Herculean task. It's not as if you can give a mouse an algebra problem or ask a dog to recite Shakespeare. To answer this, we must wind the clock back to the 1890s.

Ivan Petrovich Pavlov was a Soviet experimental neuroscientist hailing from Saint Petersburg State University; born from his work was the concept of classical conditioning [10]. Pavlov is colloquially famous for his observation of reflexive salvation (which he hilariously referred to as "psychic secretions") in dogs when presented with food. In this case, the food is the unconditioned stimulus (US) and the salivation reflex is the unconditioned response (UR). If a neutral stimulus (NS), such as a bell, were to be repeatedly presented with the US, the dog would gradually begin to associate the bell with the arrival of food. The bell has now transitioned into a conditioned stimulus (CS) and will elicit a consummatory, conditioned response (CR). The learned association between US and CS is a reflexive one, and can thus be leveraged as an experimental tool. Within the classical conditioning paradigm, researchers wouldn't have to ask questions to determine whether an animal effectively learned, just sound a bell.

As of recent, classical conditioning has quickly become the new norm for studies of sleep deprivation. The process is quite simple and tremendously reliable: (1) form an association between a US and CS of choice, (2) keep the animal awake, and (3) test for the pairing of US and CS by looking for the CR (4) profit." One such study put this exact paradigm to the test in a rodent model, attempting to observe whether acute sleep deprivation impairs the formation of a classical association between some sweet, delicious juice (US) and a flash of intense light (CS). This particular study found that sleep-deprived

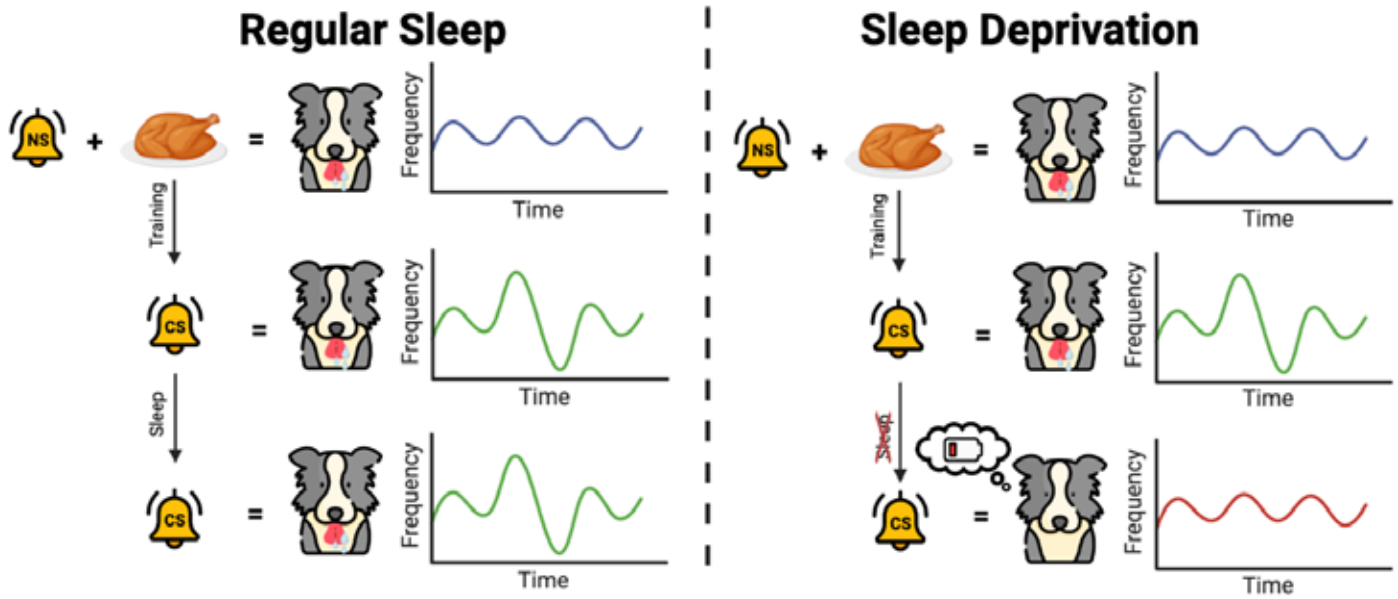
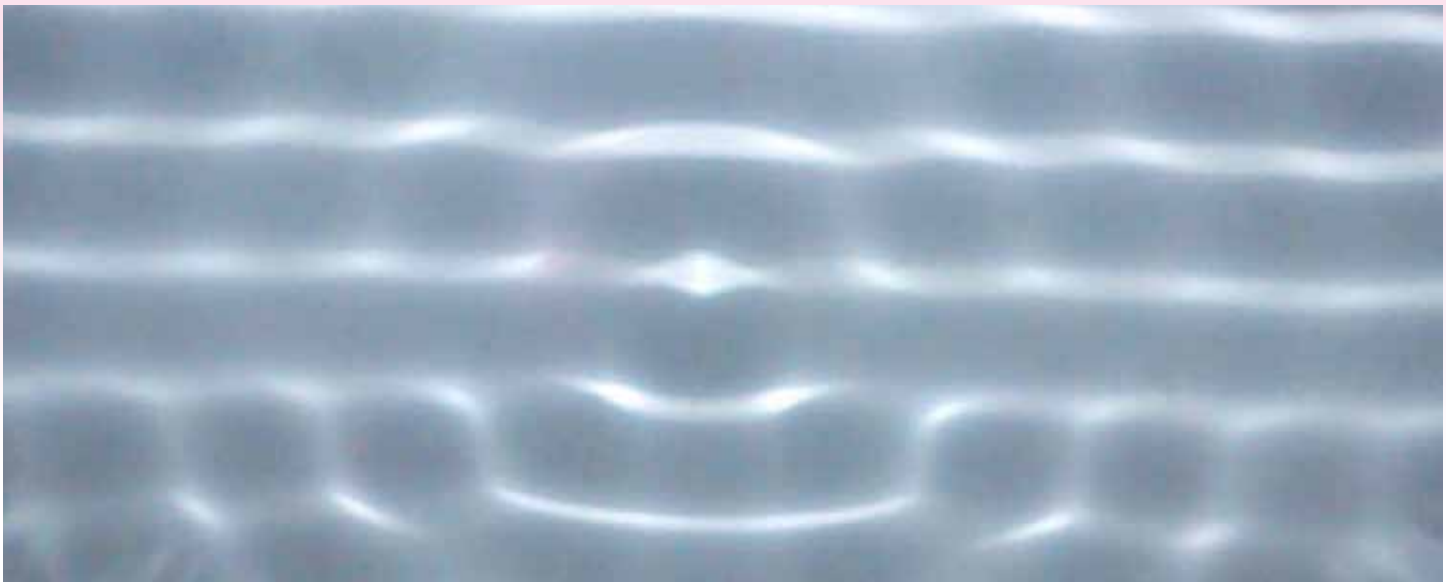


Figure 2: *Sleep deprivation inhibits memory consolidation within the classical conditioning paradigm. Created in Biorender; designed using images from Flaticon.com: Pavlov's Dog Needs Sleep Too by Maximos P. McCune under CC BY 4.0.*



rats took longer to form the association between the juice and the light, validating the use of the Pavlovian conditioning paradigm for this application [11]. Given this, we now have a method for inducing sleep deprivation and quantifying its effects—but the physiological effects of a poor night's rest remain a mystery. How exactly does a lack of sufficient sleep elicit such effects in the brain?

Practice Makes Perfect, and the Brain is no Different

The basic, time-tested principles of synaptic plasticity, the ability of neurons to change structurally and functionally in response to experience, was first established in the early 1940s. They told us that the repeated action of neurons is crucial for memory consolidation; but the underlying mechanism eluded scientists for quite some time [12] [13],[14]. We've all heard the age-old axiom: "practice makes perfect," and can imagine how this applies to the world of team sports or playing in a band. But how does the brain practice? When does the brain practice? Who are the members of "team brain?"

Hypothetically, let's assume that you learned to swing a tennis racket, and this action required only three neurons: neuron 1, neuron 2, neuron 3 (a gross oversimplification to be sure, but it serves to illustrate a point). To perform this action, the neurons must fire 1, then 2, then 3; this exact order results in a swing that would make Roger Federer jealous. To deposit the new swing into long-term memory, the firing pattern must be repeated over and over again—strengthening the ordered connection between the three neurons. In other words, the more a group of neurons fire together—the stronger the connection becomes. During sleep, this wave of neuronal firing is continuously replicated, further reinforcing the memory through synaptic plasticity. As a result, the memory is stronger when you wake up after a long tennis practice than it was before you went to sleep [15]. But is it possible that this stereotyped firing of neuronal ensembles is disrupted in conditions of poor sleep? Are your hours of tennis practice all in vain if you stay up late scrolling through YouTube or browsing Netflix?

Sharp-wave Ripples: What are they and why do they matter?

During periods of wakefulness, we experience the world through a ceaseless stream of sensory input. We're constantly bombarded with information throughout the day, and it's likely not until we sleep that our brains get a chance to engage in some of the processes that construct memories from this new information. When we sleep, the processes that drive memory formation are activated, initiating patterned spikes in electrical activity [5]. With advances in polysomnography, which take advantage of electroencephalograms (EEG) or intracranial electrodes, researchers have attempted to track and characterize the various cranial waveforms displayed during sleep epochs. Specifically, much of this work has honed in on the hippocampus (a region of the brain typically associated with learning and memory), and whether transient hippocam-

Figure 3: *Sharp wave ripples in the hippocampus facilitate cross-talk between brain regions and the formation of context-dependent memory. Created in Biorender; designed using images from Flaticon.com: Brain Cross-Talk by Maximos P. McCune under CC BY 4.0.*

pal oscillations can provide insight into the operations that underlie sleep-facilitated memory formation [6]. By combining our knowledge of brain region-specific function and targeted recordings of neural activity, scientists have made advances in understanding how memories are created and reinforced in the sleeping brain

Sharp-wave ripples are almost always observed in hippocampal neurons during non-rapid eye movement sleep (a stage of sleep marked by slowed, synchronous breathing, heart rate, and cortical firing rate) [6]. These ebbing tides of neural activity begin in the deepest of the hippocampus' three layers: CA3. Here, a cluster of neurons are spontaneously activated—manifesting as sharp, 100 to 250 Hz spikes on an EEG reading [5]. The dominant theory in the field, the two-stage theory [15], postulates that the firing of CA3 cells generates subsequent activation of the more superficial hippocampal regions (CA2 and CA1). You can picture this almost as if the CA3 neurons are sparks, which light a fuse of CA2 interneurons before igniting a firework of CA1 cells that burst into the mental images that we know as memories (Figure 1) [5]. Interestingly, the brain preferentially reactivates CA3 assemblies that were firing during the most recent wake period. This suggests that the brain "practices" while you sleep by refiring specific neural circuits. Subsequent studies took this a step further, and showed that the hippocampus repeats the exact firing sequences from a prior day's learning task when asleep [16]. The reactivation of sharp-wave ripple events in the hippocampus have even been observed in humans [4]; this further articulates the possibility of a replay mechanism at work. In essence, sharp-wave ripples serve as a cognitive biomarker for learning and memory [17]. But imagine if these brain waves occurred at the same speed as they do during the day? It would take an eternity to commit anything to long-term memory, as we are awake for plenty more hours than we sleep. There simply isn't enough time! Luckily, sharp-wave ripples are temporally compressed [17]. Temporal compression is a term used in the scientific literature to describe the speed of neural firing; it implies that a group of neurons are firing in the same pattern but much faster. This is both convenient and fits the narrative that sharp-wave ripples are a form of practice for the brain. The accelerated firing rate of sharp-wave ripples explains how your brain can replay 16 hours of footage from the prior day in just half as many hours.

As mentioned previously, repeated firing of the same neurons strengthens the connection of that circuitry. It's like a mailman traveling down a well-paved highway versus an off road trail—information travels faster down the more established routes of neural processing. The question now is whether a poor night of sleep ruins the initiation of sharp-wave ripples. Well, grab your sleep masks and earplugs because yes… it turns out that it does.

Sharp-wave Ripples are Blocked by Sleepless Nights

Remember Pavlov's dog from earlier? Well, a 2022 research paper titled: "Sleep Deprivation Impairs Learning-Induced Increase in Hippocampal Sharp Wave Ripples and Associated Spike Dynamics during Recovery Sleep," used the classical conditioning + sleep deprivation experimental paradigm to test whether a lack of sleep blocks the formation of new long term-memories [6]. To quickly reiterate the experimental procedure (as depicted in Figure 2): a paired association is formed between a NS and US in both treatment groups. In one group,

the test subjects were allowed a full night's rest between training and testing periods. In the other, subjects were kept awake to induce sleep deprivation. Specifically, this study implemented one day of sleep deprivation in healthy specimens as a way to proxy acute sleep loss. Following the training and sleep periods, the NS (now CS) should remain after a full night's sleep. In conditions of sleep deprivation, however, the pairing of NS and US should be more fragile, in that the pairing takes longer to form and is rapidly extinguished after an association is eventually made. This study expanded the classical conditioning method, choosing to integrate modern EEG technology in an effort to pair sleep-mediated learning with the underlying neural circuitry.

What this study discovered was quite astonishing! By tracking the occurrence of sharp-wave ripples before and after establishing the CS in well-slept and sleep deprived specimens, the researchers found that sharp-wave ripples were severely hindered in conditions of poor sleep. To counter, as illustrated in Figure 2, sharp-wave ripple waveforms that were generated during training persisted after a night of regular sleep. This further belabors the idea that the brain works to consolidate new memories during non-rapid eye movement sleep. That's cool and all, but what about the waveforms? We expect poor sleep to decrease learning abilities, but what of the sharp-wave ripples? Provided that each sharp-wave ripple firing pattern is indicative of very stereotyped neuronal activation, how do sharp-wave ripple waveforms reflect the macroscopically observable changes in learning?

Interestingly enough, sharp-wave ripples indicative of NS and US association were abolished after sleep deprivation [6]. The sleep deprived group not only failed to show the reflexive association after one sleepless night, but also exhibited sharp-wave ripple firing patterns that mirrored that of an untrained individual. So even at the level of synaptic plasticity and neural wiring, sleep deprivation blocks the replay of hippocampal CA3 → CA2 → CA1 cells. And just to reiterate, the sleep deprived individuals failed to display a reflex to the CS after just one night without sleep—even if the association was observed one day prior. The reflex didn't consolidate into stable long-term memories without proper sleep, implying that sleep is necessary for initiation of sharp-wave ripples. These findings suggest that a single day of sleep loss can have profound effects on the ability to form new memories. Further, older and otherwise established memories also showed diminished sharp-wave ripple reinforcement under conditions of insufficient sleep. By testing the sharp-wave ripple firing patterns of pre-learned NS/US pairings, scientists worked to show that the upkeep of past memories might also be affected by a lack of sufficient sleep. The evidence provided by these studies suggest that sleep is necessary for both the consolidation of new memories and the strengthening of old neural connections. Long story short: get your 8 hours of sleep, everyone.

Message in a Bottle: Sharp-wave Ripples are Used to Send Messages across the Brain

So we now know that sharp-wave ripples are important for memory consolidation in the hippocampus, but what of other brain regions? It turns out that sharp-wave ripples also have an impact on alternative cortical and subcortical targets [15]. The waves that originate in CA3 neurons flow up through the hippocampus and continue on to other regions of the brain [17]. Recent research efforts suggest that hippocampal sharp-wave ripples excite neurons throughout the brain and work to form context-dependent memory. On the surface, it might make sense that the amygdala and locus coeruleus (two brain regions commonly associated with fear and fear response) would activate in scary or threatening situations. Let's imagine that a person who is deathly afraid of clowns watches Andrés Muschietti's 2017 hit film "It" or finds themself at a heavily staffed carnival. That person might find that their breathing gets heavy, heart rate skyrockets, and pupils dilate to the size of nickels. This is the autonomic nervous system hard at work in response to signals from the amygdala and locus coeruleus. But for this to happen, the person must remember that they are afraid of clowns. Therefore, there must exist a connection between the hippocampus and the fear-associated brain regions [18].

Get Your 8 hours...Doctor's Orders

Harkening back to our previous debate as to whether the benefits of a good night's rest do indeed outweigh the potential for extra studying or that one extra episode of your favorite TV show;

it seems that science has a definitive answer. Sleep is not only beneficial, but necessary for memory consolidation. Without sleep, it's quite possible that the all-nighter you pulled to cram extra practice questions for tomorrow's exam was all in all vain—as you might not remember them anyways. In our discussion of sharp-wave ripples, we saw that these brain waves echo pre-established patterns from daytime events. Like with the tennis example from earlier, our brains are hard at work when we sleep, reinforcing information and skills we acquire throughout the day. Neurons that spend more time practicing together tend to perform more efficiently, and the brain is aware of this. During non-rapid eye movement sleep, the neurons in your hippocampus rehearse the coordinated dances learned the day prior, ingraining your memories deeper and deeper. So next time you are considering a late night coffee run or downing an energy drink of choice, remember that you are also potentially waving goodbye to some good memories.

References

1. H. G. Lund, B. D. Reider, A. B. Whiting, and J. R. Prichard, "Sleep patterns and predictors of disturbed sleep in a large population of college students.," J. Adolesc. Health, vol. 46, no. 2, pp. 124–132, Feb. 2010, doi: 10.1016/j.jadohealth.2009.06.016.
[2] W.-L. Chen and J.-H. Chen, "Consequences of inadequate sleep during the college years: Sleep deprivation, grade point average, and college graduation.," Prev. Med., vol. 124, pp. 23–28, Jul. 2019, doi: 10.1016/j.ypmed.2019.04.017.
[3] M. Louca and M. A. Short, "The effect of one night's sleep deprivation on adolescent neurobehavioral performance.," Sleep, vol. 37, no. 11, pp. 1799–1807, Nov. 2014, doi: 10.5665/sleep.4174.
[4] T. Schreiner and T. Staudigl, "Electrophysiological signatures of memory reactivation in humans.," Philos. Trans. R. Soc. Lond. B Biol. Sci., vol. 375, no. 1799, p. 20190293, May 2020, doi: 10.1098/rstb.2019.0293.
[5] G. Girardeau and V. Lopes-Dos-Santos, "Brain neural patterns and the memory function of sleep.," Science, vol. 374, no. 6567, pp. 560–564, Oct. 2021, doi: 10.1126/science.abi8370.
[6] R.-R. Li et al., "Sleep Deprivation Impairs Learning-Induced Increase in Hippocampal Sharp Wave Ripples and Associated Spike Dynamics during Recovery Sleep.," Cereb. Cortex, vol. 32, no. 4, pp. 824–838, Feb. 2022, doi: 10.1093/cercor/bhab247.
[7] H. Miyawaki and K. Diba, "Regulation of Hippocampal Firing by Network Oscillations during Sleep.," Curr. Biol., vol. 26, no. 7, pp. 893–902, Apr. 2016, doi: 10.1016/j.cub.2016.02.024.
[8] D. Levenstein, B. O. Watson, J. Rinzel, and G. Buzsáki, "Sleep regulation of the distribution of cortical firing rates.," Curr. Opin. Neurobiol., vol. 44, pp. 34–42, Jun. 2017, doi: 10.1016/j.conb.2017.02.013.
[9] A. Aleman-Zapata, R. G. M. Morris, and L. Genzel, "Sleep deprivation and hippocampal ripple disruption after one-session learning eliminate memory expression the next day.," Proc Natl Acad Sci USA, vol. 119, no. 44, p. e2123424119, Nov. 2022, doi: 10.1073/pnas.2123424119.
[10] W. Brown, "Conditioned Reflexes. By I. P. Pavlov. Translated and edited by G. V. AnrepM.D., D.Sc., (Oxford University Press: Humphrey Milford. 1927. Pp. xv + 430. Price 28s.)," Philosophy, vol. 3, no. 11, pp. 380–383, Jul. 1928, doi: 10.1017/S0031819100028242.
[11] Y. Stussi, A. Ferrero, G. Pourtois, and D. Sander, "Achievement motivation modulates Pavlovian aversive conditioning to goal-relevant stimuli.," NPJ Sci. Learn., vol. 4, p. 4, Apr. 2019, doi: 10.1038/s41539-019-0043-3.
[12] O. F. Hutter, "Post-tetanic restoration of neuromuscular transmission blocked by D-tubocurarine.," J Physiol (Lond), vol. 118, no. 2, pp. 216–227, Oct. 1952, doi: 10.1113/jphysiol.1952.sp004788.
[13] R. S. Zucker and W. G. Regehr, "Short-term synaptic plasticity.," Annu. Rev. Physiol., vol. 64, pp. 355–405, 2002, doi: 10.1146/annurev.physiol.64.092501.114547.
[14] M. Kordas, "A study of the end-plate potential in sodium deficient solution.," J Physiol (Lond), vol. 198, no. 1, pp. 81–90, Sep. 1968, doi: 10.1113/jphysiol.1968.sp008594.
[15] G. Buzsáki, "Two-stage model of memory trace formation: a role for 'noisy' brain states.," Neuroscience, vol. 31, no. 3, pp. 551–570, 1989, doi: 10.1016/0306-4522(89)90423-5.
[16] B. E. Pfeiffer, "The content of hippocampal 'replay'.," Hippocampus, vol. 30, no. 1, pp. 6–18, Jan. 2020, doi: 10.1002/hipo.22824.
[17] G. Buzsáki, "Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning.," Hippocampus, vol. 25, no. 10, pp. 1073–1188, Oct. 2015, doi: 10.1002/hipo.22488.
[18] A. Choucry, K. Ghandour, and K. Inokuchi, "The locus coeruleus as a regulator of memory linking.," Neuron, vol. 110, no. 20, pp. 3227–3229, Oct. 2022, doi: 10.1016/j.neuron.2022.09.014.