- Common ancestor of humans and Drosophila lived around 800 million years ago [12]
- Studying less complex brain of Drosophila melanogaster led to a deeper understangind of the neural principles of sleep regulation [11] (Suarez et al 2021)
- Slow wave activity provides the neurophysiological basis to establish a sensory gate that suppreses sensory processing to provide a resting phase which promotes synaptic rescaling and clearance of metabolites from the brain [11] (Suarez et al 2021)
- Evolutionary adaptation to day/night - Hunting and resting [11] (Suarez et al 2021)
- Distored balance between waking and sleep has been linked to various health conditions, like hypertension, diabetes, depression and deficits in attention and learning (Alhola andPolo-Kantola 2007, Mullington et al 2021) [11] (Suarez et al 2021)
- Common between spicies: SWA resulting from large scale synchronization -¿ reduction of functional connectivity -¿ low arousability; [11] (Suarez et al 2021)
- Differences: For some birds and marine mammals one half of the brain generates SWA, while other half remains more easily arousable, allowing the sleeping animal to maintain a certain level of alertness [11] (Suarez et al 2021)
- In mammals, deep sleep occurring during NREM sleep is characterized by a particularly high arousal threshold and slow-wave activity (up to 4.5 Hz) resulting from cortical neurons synchronizing their electrical patterns (Bonnet et al 1978, Buzsaki and Draguhn 2004) [11] (Suarez et al 2021)
- Slow-wave sleep has been shown to be important for memory consolidation (Klinzing et al 2019), and the clearance of metabolites (Fultz et al 2019), which would otherwise accumulate and increase the susceptibility for neurodegenerative diseases [11] (Suarez et al 2021)
- Mechanisms must exist for specific sensory signatures to awaken us even from our deepest slumber. How neural networks can differentiate sensory stimuli and mediate awakening from deep sleep is very complex and far from understood [11] (Suarez et al 2021)
- Evolutionary distant brains, to probe for fundamental processes regulating sleep and wakefulness [11] (Suarez et al 2021)

- Cognitive impariments in sleep-deprived humans have been linked to local synchronization of SWA, indicating a role for SWA in shutting down sensory processing and signaling sleep need to undergo synaptic plasticity (Quercia et al 2018) [11] (Suarez et al 2021)
- Sleep-like state indicates that slow wave sleep might be an inheret property of neural networks (Krueger et al 2008). In fact, even isolated cortical slices retain the ability to generate synchronized SWA (Sanchez-Vuves 2020) [11] (Suarez et al 2021)
- In Drosophila, synchronized SWA does not only mediate sleep need but also fascilitates consolidates sleep phases and decreases arousability during the night [8, 11] (Suarez et al 2021)
- In 2017 the Nobel prize in hysiology was awarded to the Drosophila researchers that made significant contributions to understanding the modelcular clockwork of circadian rhythms (Hardin et al 1990, Zehring et al 1984) [11] (Suarez et al 2021)
- The transciptional and translational feedback loops that represent the mechanistic basis for generating circadian rhythms at the cellular level are found in all animals and plants [11] (Suarez et al 2021)
- Ensuring tha sleep always occurs during the same phase of the day [11] (Suarez et al 2021)
- Fruit flies are mostly active during dusk and dawn, having an extended period of rest in the middle of the day and exhibiting deeper sleep throughtout the night (Shaw et al 2000) [11] (Suarez et al 2021)
- (In Drosophila) Transition frin wajubg ti skeeo has been assicuated with an increase in oscillatory activity in the centran brain (Yap et al 2017) [11] (Suarez et al 2021)
- As in humans, sleep deprivation leads to deficits in selective visual attention (Kirszenblat et al 2018) and memory performance (Donela 2019) as well as to the accumulation of metabolites in the brain (van Alphen et al 2021) [11] (Suarez et al 2021)
- (Dorosphila) Neural networks involved in mediating hunber and sexual arousal reduce sleep while neural networks processing visual information and social cues have been shown to be sleep-promoting. The mushroom bodies, a higher-order brain network curcially involved in olfactory memore in Drosophila, have also been implicated in sleep regulation. Some mushroom body neurons have been shown to be wake-promoting, while others seem sleep-promoting [11] (Suarez et al 2021)

- It is within the central complex that we find an oscillatory activity that is tightly linked to sleep need and sleep quality [8, 11] (Suarez et al 2021)

- In fruit files caffeine promotes wakefulness by directly activating dopaminergic neurons (Nall et al 2016) [11], impressiely demonstraing that humans and flies must share ancient neural mechanisms for homeostatic sleep regulation [11] (Cite first source + one about humans) (Suarez et al 2021)

- The dFSB is often referred to as "master sleep switch" because its optogenetic activation ha been tightly linked to reducing arousability (Tropup et al 2018) and inducing sleep (Donela et al 2009, Pimentel et al 2019). During course of the day activity-dependent build-up of ROS in dFSB neurons increases their excitability (Kempf et al 2019), leading to increased inhibition of the helicon cells during the night (Donela et al 2018). Helicon cells process light cues to induce locomotion and their inhibition therefore reduces the flow of visual information and suppress locomotion (Donela et al 2018). Here, R5 ring neurons, which have reciprocal synaptic connections to the helicon cells provide another layer of homeostatic sleep regulation that is directly linked to pricessing compex visual and orientation behavior [11] (Suarez et al 2021)

- R5 neurons display intriguing functional and physiological analogies to the mammalian thalamus. Thalamus can filter sensory information, essentially acting as a sensory gate that regulates sleep/wake transitions (Gent et al 2018). As sleep need increases, thalamic reticular neurons switch from tonic firing to bursting and promote network synchronization that generates SWA and facilitates the transition to sleep (Llinas and Steriade 2006), Similarly, with increasing sleep need, R5 neurons start bursting and synchronize their electrical patterns, generating NMDA receptor-dependent compound SWA (Liu et al 2016, Raccuglia et al 2019) [11] (Suarez et al 2021)

- Similar to the thalamus or the local sleep phenomenon, network-specific slow-wave synchronizations in Drosophila can establih a gate at the level of neural networks that suppresses sensory processing and mediates sleep need [11] (Suarez et al 2021)

- Sleep need and subjectie tiredness also need to be regulated by other internal drives such as hunger and sexual arousal, which generally are wake-promoting [11] (Suarez et al 2021)

- Homeostatic sleep need in mammals and flies is genearted by an accumulation of byproducts of neural acivity, which often correlates with the amount fond compexity of sensory information [11] (Suarez et al 2021)

- In Drosophila, spatial learning depending on ring neurons has been shown to deteriorate in sleep-deprived flies (Melnattur et al 2020, Neuster et al 2008) [11]. (Suarez et al 2021)
- Spatial information could ”accumulate” in ring neurons to the point where they switch from processing sensory information to mediating sleep need which might require R5 neurons to start bursting and synchronize their electrical patterns (Liu et al 2016, Raccuglia et al 2019) [11] (Suarez et al 2021)
- Prolonged optogenetic activation of TuBu neurons leads to synchronized SWA in R5 neurons and dramatically increases sleep (Guo et al 2018, Raccuglia et al 2019), illustrating the link between visual processing, network synchronization and sleep need [11] (Suarez et al 2021)
- As our molecular clocks are not precisely running on a 24 h cycle, so-called clock neurons need light input to constantly reset the molecular clocks (Dunlap 1999) [11] (Suarez et al 2021)
- Dorso-posterior clock nerons (DN1p) provide circadian information to the TuBu neurons, illustrating two important aspects of circadian retulation (Guo et al 2018) [11] (Suarez et al 2021)
- Circadian time influences sensory processing. Secondly, the circadian time could determine when and to what extent sensory processing leads to the accumulation of sleep need or subjective tiredness [11] (Suarez et al 2021)
- Circadian and homeostatic sleep regulation have coevolved and are thus inexorabl linked to ech other [11] (Suarez et al 2021)
- Optigenetic activation of DN1p clock neurons leads to oscillatory activity in ring neurons, demonstraing the circadian influence of generating sleep need at the level of neural networks (Guo et al 2018) [11] (Suarez et al 2021)
- Circadian input to visually sensitive TuBu neurons might promote sensory processing during the day while facilitating the switch of R5 neurons from tonic firing to bursting in the night [11] (Suarez et al 2021)
- TuBu neurons provide parallel synaptic input to helicon cells and R5 neurons (Hulse et al 2020). While R5 neurons might use this visual information for navigation, light information in helicon cells might be decisive whether locomotion is induced or not. Interacions between the helicon cells and R5 neurons could therefore integrate use-dependent sleep need, the presence of absence of light and the circadian time. [11] (Suarez et al 2021)

- We identify a subset of EB neurons whose activation generates sleep drive [6] (Liu et al 2016)
- Elevated sleep need triggers reversible increases in cytosolic Ca levels, NMDA expresion and structural markers of synaptic strength, suggesting these EB neurons undergo sleep-need-dependent plasticity [6] (Liu et al 2016)
- Synaptic plasticity of these EB neurons is both necessary and suffucuent for generating sleep drive [6] (Liu et al 2016)
- Substantial accumulation of sleep drive in many animals takes hours of wakefulness and is often maintained for hours even after sleep is initiated (Daan et al 1984, Huber et al 2000, 2004) [6] (see - what is meant by accumulation) (Liu et al 2016)
- From an engineering perspective, hoeostatic systems require three components: 1) sensor that periodically samples the state variable 2) an integrator that processes this information to determine homeostatic drive and 3) an effector that responds to this drive by directly manipulating the state variable (Enderle and Bronzino 2012) [6] (Liu et al 2016)
- Drosophila is an established genetic model system for studying sleep and shares similar sleep-regulatory mechanisms with vertebrates (Cirelli 2009, Sehgal and Mignot 2011). For example, sleep in fruit flies is regulated by both the circadian clock and homeostatic sleep drive (Donela et al 2014, Kunst et al 2014, Liu et al 2014) [6] (Liu et al 2016)
- Data suggests that activating R2 neurons genetically induces persistent sleep drive [6] (Liu et al 2016)
- Inhibition of neurotransmitter release reduced ”rebound sleep” as well as sleep depth following mechanical sleep deprivation (R2 neurons are required for generating homeostatic sleep drive) [6] (Liu et al 2016)
- Inactivation of R2 neurons did not significantly affect baseline sleep, indicating a specific role for these neurons in homeostatic regulation of sleep [6] (Liu et al 2016)
- Under baseline conditions, we recorded from two time points: ZT0-ZT2 (when speel pressure should be low) and TZ13-ZT15 when sleep pressure should be moderately elevated. We found that spontaneous AP firing rate of R2 neurons was increased 3fold at ZT13-15, compared ZT0-2. Moreover, following sleep deprivaion, the spontaneous AP firing rate further increased, compared

to rested flies assessed at ZT0-2. Strikingly, bursting activity was were seen in the majority of recordings from sleep-deprived flies, but never seen under baseline conditions at ZT0-2 or ZT13-15, suggesting a switch to a potentiated state after sleep loss. [6] (Liu et al 2016)

- The resting membrane potential of R2 neurons was also more depolarized in animals following sleep deprivation, while input resistance was not altered [6] (Liu et al 2016)
- Data indicate that the activity and excitability of the R2 circuit increase under conditions of greater sleep need and that these neurons exhibit burst firing specifically following sleep deprivation [6] (Liu et al 2016)
- Two hours after mechanical sleep deprivation had ended, BRO signal in R2 ring structure was significantly increased in sleep-deprived flies compared to rested control flies, and this greater BRP signal was due to increases in both the number and size of BRP puncta in the R2 ring [6] (Liu et al 2016)
- When sleep drive is dissipated following 26 h of recovery sleep, increased BRP signal is lowered to levels seen in rested control animals -¿ these changes are reversible. Increased BRP signal following sleep deprivation was not observed in other neurons in the brain [6] (Liu et al 2016)
- The data argue that levels of molecular markers of synaptic strength in R2 neurons correlate with levels of sleep drive and suggest that "sleep-need"-dependent plastic changes in the R2 circuit may be relevant for generating homeostatic sleep drive [6] (Liu et al 2016)
- Data suggest, that Ca levels are specifically increased within R2 neurons following sleep deprivation (For other neurons it was unchanged) [6] (Liu et al 2016)
- Ca levels in the R2 neurons correlate with varying levels of sleep drive in a scalable manner [6] (Liu et al 2016)
- Blocking the rise of intracellular Ca levels in R2 neurons significantly impared the increases in number and size of BRP puncta seen in these neurons following sleep deprivation [6]. Ok, but what happens to synchronization? And bursting? Not stated in the paper (Liu et al 2016)
- These data suggest that Ca-dependent plastic changes in R2 neurons are specifically required for proper homeostatic regulation of sleep [6] (Liu et al 2016)

- Increasing temperature for animals expressing dTrpA1 in R2 neurons led to an increase in number and size of BRP puncta, similar to that seen following sleep deprivation, suggesting increase in synaptic strength. Moreover, as little as 30-min activation of dTrpA1 in the R2 circuit inducted a significant increase in sleep mimicking "rebound sleep" following the temperature elevation, despite the animals being fully rested. Thus, these data suggest that Ca-dependent changes in synaptic strength of the R2 circuit are not only necessary, but also sufficient for encoding sleep drive [6]. (If dTrpA1 are non-selective cation channels, why increasing temperature would increase Ca concentration?) (Liu et al 2016)
- Activation of R2 neurons generates sleep drive even in fully rested animals [6] (Liu et al 2016)
- While R2 neurons play a crucial role in the homeostatic regulation of sleep following sleep deprivation, additional mechanisms likely play a role under baseline conditions [6] (Liu et al 2016)
- We propose that it is encoded by Ca and NMDA-receptor dependent plastic changes in a dedicated neural circuit [6] (Liu et al 2016)
- (Okay, so, if Ca concentration affects the bursting, maybe one should model Ca dependent channels with Goldman-Hodgkin-Katz flux equation ??? It is even second argument for this equation, the first one being that the fit is better!!!) (Liu et al 2016)
- Sleep-wake cycle is determined by circadian and sleep homeostatic processes [3] (Dopp et al 2024)
- Cell type-specific transciptomic changes, with glia displaying the largest variation [3] (Dopp et al 2024)
- Glia are also among the few cell types whose gene expression correlates with both sleep hoeostat and circadian clock [3] (Dopp et al 2024)
- Sleep is regulated by two independent processes: the circadian system and the sleep homeostatic system [3] (Dopp et al 2024)
- Circadian clock primarily regulates the timing of sleep, known as process C. It consists of a transciptomial-translational feedback loop of core clock genes. However, it is unclear whether and how process C affects the transciptomes of any given brain cell population apart from pacemaker regions and neurons [3] (Dopp et al 2024)

- The sleep homeostat monitors the sleep need that accumulates with the amount of time that an animal has been awake to determine the sleep drive, known as process S [3] (Dopp et al 2024)
- We found that sleep/wakefulness states, sleep homeostasis and circadian rhythm have different transcriptional correlates depending on cell identity [3] (Dopp et al 2024)
- Gene expression in most cell populations correlates either with process C or process S, with the exception of glial cells, which instead are affeced by both processes simultaneously [3] (Dopp et al 2024)
- Cell types involved in sleep: five glial cell subtypes, Kenyon cells (KCs), clock neurons and cell types containing known sleep/wakefullness regulating curcuits such as non-protocerebral anterior medial (PAM) dopaminergic neurons (DANs), tyraminergic (Tyr) and octopaminergic (Oct) neurons, and ellipsoid body (EB) ring neurons. Another tell type involved in sleep, the dorsal fan-shaped bidy (dFB) neuron... [3] (Dopp et al 2024)
- period (per) and timeless (tim) - expressed at higher levels in the early night compared to the early day; cryptochrome (cry) and Clock (Clk) mRNA - the opposite. Core clock genes are expressed and cycle specifically in Drosophila clock neurons and glia. While Clk expression is restricted to clock neurons and glia, other core clock genes per, tim and Cycle (Cyc) are expressed in more cell types. No cell type expresses Clk without expression of other core circadian genes is consistent with the notion that Clk is a circadian master regulator [3]. (Dopp et al 2024)
- Molecular clock runs with different phases depending on the cell type [3] (Dopp et al 2024)
- The expression of key clock genes in glia in addition to clock neurons, suggests that these cells are directly involved in circadian regulation of rhythmic behaviors, including sleep. [3] (Dopp et al 2024)
- Glial cells stand out, this time by showing the highest number of cyclers (transcipts), especially considering that they typically express fewer genes than neurons [3] (Dopp et al 2024)
- KCs and glia had the highest number of differentially expressed genes among annotated cell types. Considering that glia express the lowest number of genes among all cell types, these cells may be even more affected by sleep/wakefullness relative to their total expressed genes compared to neurons [3] (Dopp et al 2024)
- Different cell populations have different sleep/wakefullness correlates [3] (Dopp et al 2024)

- The sleep/wakefullness correlates in glia included metabolism related genes, genes involved in protein synthesis and homeostasis, and genes regulating the core circadian clock. In contrast, sleep/wakefullness correlates in KCs included many genes involved in axon and synapse development and function. (Dopp et al 2024)

- Cell types involved in process S: the four annotated clusters with the highest amount of sleep drive correlates were cell populations associated with sleep homeostasis. 121 correlates by dFB neurons. Similarly, Oct, Tyr and non-PAM DAN neurons each had more than 100 sleep drive corelates. In contrast, the related dopaminergic subtype of PAM neurons only had 14 correlates. (Dopp et al 2024)

- (!!!!) In dFB neurons, we found many sleep drive correlates that were involved in synaptic formation and function. This is consistent with previous evidence linking neuronal activity of dFB neurons to levels of sleep pressure (Dopp et al 2024)

- R5 neurons contain only approximately 32 cells in an adult fly brain. (Dopp et al 2024)

- We found that one subcluster of EB ring neurons (ring_2) had a substantial number of sleep drive correlates, while the other (ring_1) showed only a few. We found a high number of sleep drive-correlated genes specifically in R5 neurons, while few to no genes were identified in the other two subclusters. (Dopp et al 2024)

- We found, that a gene encoding a potassium channel ether-a-go-go (eag) correlated negatively with sleep drive in R5 neurons. Potassium channels, including Eag, reduce neuronal excitability (**Brüggemann et al 1993**) This is consistent with the finding that the neuronal activity of R5 increases with the levels of sleep drive (Liu et al 2016). (Dopp et al 2024)

- Clock neurons are a heterogeneous population consisting of four dorsal neuron subtypes (DN1a, DN1p, DN2 and DN3) and three lateral neuron (LN) types (LNv, LNd and LPN). In clock neuron subclusters... We identified significant sleep drive correlates only in the glutamatergic-positive cluster, not in the glutamatergic-negative subtype nor in DN3 neurons. This is consistent with findings that glutamatergic DN1p neurons are involved in sleep/wakefullness regulation. Among the sleep drive correlates of the DN1p cluster are two genes that regulate the function of a potassium channel: slowpoke-binding protein (slop) and dyschronic(dysc). This is consistent with the finding that the potassium channel (slowpoke) is important in glutamatergic DN1p neurons to regulate sleep quality. (Dopp et al 2024)

- In most cases we found that a given cell type was more affected by only one process (C or S). For example, dFB, Oct/Tyr, non-PAM DAN and R5 neurons had many sleep driv correlates but few circadian cyclers. On the other hand, cell types with many circadian correlates (examples listed in paper: some KC subtypes and PGs) had no sleep drive correlates (Dopp et al 2024)
- Two subtypes of EB ring neurons, that is R5 and ring_B were affected by either processes in opposing ways, in accordance with previous findings, showing that R2/R4m neurons (probably part of ring_B) received circadian timing information from clock neurons, while **R5 neurons themselves encoded the sleep homeostat (Liu et al 2016)**. (Dopp et al 2024)
- Both the number of cyclers and sleep drive correlates were high in all glia with the exception of PGs. This demonstrated that a simultaneous convergence of both circadian and homeostatic processes takes place in glial cells, as their transcriptome s affected by both (Dopp et al 2024)
- Flies with disrupted glial clock showed significantly reduced rebound sleep after SD compared to control flies (Dopp et al 2024)
- These data indicate that the glial clock is required for normal sleep homeostasis and suggest that processes S and C directly influence each other in glial cells to determin sleep-wake cycles (Dopp et al 2024)
- Our findings of sleep drive correlates illustrate the high specificity of the method to identify relevant sleep homeostasis regulating circuits, even when they are small (sub)populations. Therefore, other yet unannotated clusters with a high number of sleep drive correlates may also be involved in the homeostatic regulation of sleep. (Dopp et al 2024)
- We propose that the sleep–wake cycle affects the regulators of core clock genes in glia, and that the molecular clock in glia is required for sleep homeostasis. How do these two processes interact in glial cells? We and others previously demonstrated that glial Ca2+ signaling encodes the level of sleep needed18,59. In addition, Ca2+ signaling has an important role in regulating the oscillation of core clock genes, with many Ca2+ channels and transporters rhythmically expressed in mammalian clock neurons62. Thus, the reciprocal interaction between Ca2+ signaling and the molecular clock in glia may be the molecular substrate of the interaction of homeostatic and circadian processes to ultimately instruct downstream neurons and appropriate behavior. (Dopp et al 2024)
- Circadian rhythms are daily rhythms in behavior or physiology that reoccur approximately every 24 hr. Circadian rhythms can be entrained by external environmental cues (i.e., light and temperature),

but persist in the absence of these cues, with free-running periods that deviate slightly from the expected 24 hr in constant environmental conditions. [4] (Dubowy and Sehgal 2017)

- In addition to eclosion and locomotor activity, circadian rhythms also drive other aspects of physiology and behavior, including sleep and an increasingly appreciated role in metabolism. Circadian control of all of these processes relies not only on the intracellular clock, but also on networks of cells that interact to influence circadian outputs. [4] (Dubowy and Sehgal 2017)

- the two mRNAS (**per and tim**) cycle in phase and the PER and TIM proteins interact directly and affect their own transcription. [4] (Dubowy and Sehgal 2017)

- while a number of period-altering mutations have been identified in per, tim, and the relevant kinases, we still do not have a complete understanding of how the 24 hr period is generated. [4] (Dubowy and Sehgal 2017)

- Inherent to clock function in flies is a feedback loop in which the per and tim genes are expressed cyclically and negatively regulated by their own protein products (Hardin et al. 1990; Sehgal et al. 1995) The Neurospora clock is likewise comprised of a negative feedback loop generated through cyclic activity of the frequency gene product (Aronson et al. 1994). [4] (Dubowy and Sehgal 2017)

- The transcriptional mechanisms discussed earlier do not just maintain rhythmic expression of per and tim, but also drive cycling of many output genes that contain enhancer elements recognized by CLK-CYC. For example, cycling of the Nlf-1 transcript results in time-of-day dependent changes in sodium leak current that, in turn, drive rhythmic neuronal activity in a subset of clock neurons (Flourakis et al. 2015). This example shows how clock-dependent cycling transcription can be the basis for rhythms in physiology that, in this case, likely contribute to behavior. however, clocks can also drive rhythms in physiology of other cells non-cellautonomously, by controlling signaling through neuronal circuits and release of secreted factors (Jaramillo et al. 2004; Cavey et al. 2016; Erion et al. 2016). [4] (Dubowy and Sehgal 2017)

- In Drosophila, the core molecular clock components are coexpressed only in a restricted set of 150 neurons, which serve a function similar to the mammalian superchiasmatic nucleus (SCN) in regulating circadian rhythms in behavioral activity. [4] (Dubowy and Sehgal 2017)

- Clock cells also exhibit cycling neuronal activity (Cao and Nitabach 2008; Sheeba et al. 2008b; Flourakis et al. 2015), and the peak of neuronal activity, as reflected by intracellular calcium levels,

occurs at different phases for different groups (Liang et al. 2016). However, in wild-type flies kept in conditions that approximate the natural world (including LD and early DD), the core molecular clocks in nearly all groups of clock neurons cycle approximately in phase with each other (Yoshii et al. 2009a; Roberts et al. 2015). The mechanisms through which the cycling of the molecular clock generates complex and flexible behavioral outputs are thus probably related to these other properties of the clock circuit, rather than to differences in the cycling of the molecular clock itself. [4] (Dubowy and Sehgal 2017)

- An important signaling molecule necessary for keeping clock cells synchronized with each other and orchestrating behavioral activity is the neuropeptide PDF. Without PDF, the molecular clock in some groups of clock neurons run fast, while others dampen as individual cells fall out of phase with each other, and yet others may slow (Klarsfeld et al. 2004; Lin et al. 2004; Yoshii et al. 2009b; L. Zhang et al. 2010). [4] (Dubowy and Sehgal 2017)

- A molecular clock in a subset of DN1 is sufficient to drive morning anticipatory activity in LD cycles, and, in certain temperature conditions, can drive evening anticipation as well (Y. Zhang et al. 2010). [4] (Dubowy and Sehgal 2017)

- Drosophila has been essential for identifying the key clock molecules and the negative feedback loop mechanism that produces 24 hr cycles of gene expression and overt rhythms. [4] (Dubowy and Sehgal 2017)

- Importantly, many genetic and molecular regulators of sleep are conserved across species (reviewed in Crocker and Sehgal 2010). Thus, sleep in flies closely resembles sleep in other organisms, and researchers can take advantage of the benefits of this small, genetically tractable model organism to advance our understanding of the molecular neuroscience of sleep. [4] (Dubowy and Sehgal 2017)

- Based on initial studies of arousal threshold, sleep in Drosophila is commonly defined as a period of inactivity lasting 5 min or longer (Shaw et al. 2000; Huber et al. 2004; Andretic and Shaw 2005). [4] (Dubowy and Sehgal 2017)

- The Shaker potassium channel (Cirelli et al. 2005; Bushey et al. 2007) and its modulator sleepless (Koh et al. 2008) were two early hits with extreme short-sleeping phenotypes from large-scale genetic screens. Both genes are expressed throughout the fly brain (Wu et al. 2009), and neither of these phenotypes has been fully mapped to specific neuroanatomic loci, suggesting that they exert widespread effects on brain activity or metabolism that feed back onto sleep regulation. Shaker is a voltage-gated potassium channel involved in membrane repolarization. sleepless is a Ly6 neurotoxin-like molecule that, in the years since its discovery as a sleep regulator, has been found to promote Shaker expression and activity and inhibit nicotinic acetylcholine (nAChR) function, such that loss of sleepless might lead to increased neuronal activity through multiple mechanisms (Wu et al. 2009; Shi et al. 2014; Wu et al. 2014). [4] (Dubowy and Sehgal 2017)

- knocking down Shaker in sleep-promoting cells actually lengthens the inter-spike interval and reduces neuronal activity in these populations to favor wake (Pimentel et al. 2016). [4] (Dubowy and Sehgal 2017)

- Recent studies of sleepless have also suggested that it in part regulates sleep by noncell-autonomously promoting metabolism of GABA in glia, perhaps also through its effect on neural activity (Chen et al. 2014; Maguire et al. 2015). Shaker and sleepless thus both seem to interact in a nonstraightforward way with sleepregulatory genes and cells in the nervous system, and work with sleepless suggests a potential connection between neuronal activity and metabolism of neurotransmitters, although the details of this connection remain unclear. [4] (Dubowy and Sehgal 2017)

- The mushroom body consists primarily of 2000 Kenyon cells, most of which receive input from an average of six stochastically connected projection neurons, with each projection neuron encoding input from a single type of odorant receptor neuron. [4] (Dubowy and Sehgal 2017)

- The mushroom body was also the first neuroanatomic structure identified as a regulator of sleep in Drosophila (Joiner et al. 2006; Pitman et al. 2006). [4] (Dubowy and Sehgal 2017)

- mushroom body contains both sleep-promoting and wake-promoting cells [4] (Dubowy and Sehgal 2017)

- Perhaps the strongest parallel between mammalian and Drosophila sleep regulation identified so far is the strong wake promoting effects of the monoamine neurotransmitters dopamine and octopamine [4] (Dubowy and Sehgal 2017)

- dopaminergic neurons are strongly wake-promoting when activated (Shang et al. 2011; Liu et al. 2012) [4] (Dubowy and Sehgal 2017)

- Thermogenetic activation of the ExF/2 neurons in the dorsal fanshaped body, a region of the central complex, is strongly sleep-promoting (Donlea et al. 2011). Sleep deprivation changes the electrophysiologic properties of these neurons to favor activity, suggesting they may play a role in output of homeostatic sleep signals (Donlea et al. 2014). [4] (Dubowy and Sehgal 2017)

- Sleep homeostasis is often conceptualized as a continuous build-up of sleep need over periods of wakefulness and dissipation over periods of sleep, such that the same mechanisms should be invoked both when flies are spontaneously waking and during periods of forced wakefulness (sleep deprivation). However, recent work in Drosophila has called this view into question. [4] (Dubowy and Sehgal 2017)

- a number of genetic perturbations have been identified that specifically affect sleep after sleep deprivation with little to no effect on baseline sleep, suggesting that sleep following sleep deprivation is produced by an independent mechanism (Seugnet et al. 2011b; Seidner et al. 2015; Thimgan et al. 2015; Dubowy et al. 2016; Liu et al. 2016) [4] (Dubowy and Sehgal 2017)

- electrophysiology suggests that the sleep-promoting dorsal fan-shaped body neurons have reduced input resistance and reduced membrane time constants, suggesting greater activity, following sleep deprivation (Donlea et al. 2014); as discussed previously, this brain area is well-positioned to act as an integrator or output for multiple sleep regulatory signals, including, it seems, the response to sleep deprivation. [4] (Dubowy and Sehgal 2017)

- These neurons were initially of interest because they produce a persistent sleep-promoting signal when thermogenetically activated; while no changes in sleep are reported at the time of activation, which can be as short as 30 min, a dramatic reboundlike increase in sleep is observed for the next 12 hr. [4] (Dubowy and Sehgal 2017)

- showed greater synapse number and size for R2 neurons after sleep deprivation, and genetic manipulations that block this plasticity partially block sleep rebound. [4] (Dubowy and Sehgal 2017)

- The manipulations of R2 neurons that affect sleep rebound have no effect on sleep at baseline, however, again supporting the idea that regulation of the homeostatic response to sleep deprivation is mechanistically different from the regulation of baseline sleep. [4] (Dubowy and Sehgal 2017)

- Function of the sleep: bidirectional relationship with learning and memory (both short ad long-term in drosophila), social behavior [4] (Dubowy and Sehgal 2017)
- One hypothesis based on this data, put forth by Tononi and Cirelli (2006), proposes that global synaptic downscaling occurs during sleep to counteract overpotentiation that might occur during wake. Work from these authors shows evidence that, broadly and within specific circuits of the adult fly brain, synaptic markers increase after wake or sleep deprivation and decrease following sleep, suggesting changes in the number or size of synapses (Gilestro et al. 2009; Bushey et al. 2011). [4] (Dubowy and Sehgal 2017)
- This research has revealed that the functions and neural principles of sleep regulation are largely conserved from flies to mammals [10] (Shafer and Kenee 2021)
- sleep is required for numerous cellular processes that are critical for biological function, including brain-wide regulation of synaptic strength and immune function. Sleep also impacts cellular metabolism and communication between the brain and the peripheral organs it controls, as well as complex cognitive tasks, including learning and memory. [10] (Shafer and Kenee 2021)
- our understanding of the neural and molecular basis of sleep regulation and how sleep is influenced by an animal's internal and external environment remains incomplete. [10] (Shafer and Kenee 2021)
- (Drosophila studies) These efforts have revealed neural principles and molecular mechanisms that are conserved within the animal kingdom, including widespread functional conservation of the neurotransmitter systems that are primary drivers of sleep and wakefulness in mammals [10] (Shafer and Kenee 2021)
- The defining behavioral features of sleep — prolonged periods of quiescence, reduced responsiveness to sensory stimuli, species-specific posture, recovery sleep following deprivation, and rapid reversibility (which distinguishes sleep from hibernation or coma) — characterize both vertebrate and invertebrate sleep Remarkably, invertebrates with relatively simple nervous systems, including the jellyfish Cassiopea and the roundworm Caenorhabditis elegans, meet these simple criteria, suggesting deep evolutionary conservation of sleep [10] (Shafer and Kenee 2021)
- flies that are immobile for five-minute periods or longer are considered to be sleeping because inactivity for this duration is associated with the aforementioned behavioral characteristics of sleep [10] (Shafer and Kenee 2021)

- central complex is critical for the regulation of sleep duration and homeostasis. The central complex contains many neuron types, including dorsal fan-shaped body neurons, helicon cells, and ellipsoid body ring neurons, all of which have been implicated in sleep regulation. Dorsal fan-shaped body neurons are sleep-promoting neurons that receive inhibitory input from wake-promoting dopamine neurons. At the cellular level, expression levels of multiple ion channels within the dorsal fan-shaped body modulate neuronal activity and thereby promote sleep or wakefulness. [10] (Shafer and Kenee 2021)

- Specifically, a class of neurons termed the R5 neurons (originally termed R2 neurons) fire synchronously during sleep in a manner similar to neuronal firing during mammalian slow-wave sleep in the mammalian neocortex. The neural activity and synaptic connectivity of these neurons is modified in accordance with sleep debt, sensory information, and circadian input, thereby driving recovery sleep. The sleep-promoting dorsal fan-shaped body neurons interact with the R5 neurons through a third population of neurons — helicon cells — that are activated by visual stimuli. [10] (Shafer and Kenee 2021)

- The mushroom bodies are paired structures in the central brain comprising 2,500 intrinsic neurons, termed Kenyon cells, that were initially studied for their roles in learning and memory. Mushroom bodies play a central role in modulating sleep, likely through a circuit that integrates sensory information, arousal, and homeostatic sleep drive. Broad activation of the mushroom bodies promotes sleep and that sleep is reduced in flies with silenced or ablated mushroom bodies, revealing a critical role for this structure in governing sleep duration [10] (Shafer and Kenee 2021)

- dynamic changes in local field potentials and their associated changes in sleeplike behavior require gap junctions, highlighting the presence of complex network-wide signatures that may not be mimicked by the optogenetic and thermogenetic manipulations available in the fly. [10] (Shafer and Kenee 2021)

- It is clear that more work is necessary to understand how the various sleep/wake-modulating networks interact with one another. A recent connectomic reconstruction of the fly brain using serial electron microscopy will likely provide the synaptic resolution necessary to reveal the microanatomical connections between neurons within and between sleep networks, including those that regulate mushroom body and central complex physiology. [10] (Shafer and Kenee 2021)

- As the field continues to identify neurons and networks that govern sleep, it will be critical to

determine the physiological changes that occur within these networks during transitions between wakefulness and sleep, as well as during the various stages of sleep. [10] (Shafer and Kenee 2021)

- The functions and subtypes of Drosophila glia are broadly similar to those in mammals and are thought to be evolutionarily conserved. Several subtypes of glia have been implicated in the control of sleep and circadian modulation of activity. [10] (Shafer and Kenee 2021)

- Together, these observations reveal that astrocytes are critical modulators of sleep and the neural networks that govern it. [10] (Shafer and Kenee 2021)

- Taurine is a GABA agonist that promotes sleep in flies, raising the possibility that ensheathing glia promote wakefulness by removing synaptic taurine. [10] (Shafer and Kenee 2021)

- One likely function of the circadian system is to suppress the onset of sleep during times of the day when sleep pressure is high but when sleep would be dangerous or maladaptive, thereby delaying it until the appropriate time. For nearly four decades, the sleep field has conceptualized sleep through the two-process model, which posits that sleep is governed by interactions between homeostatic and circadian control processes. In this model, sustained wakefulness produces a homeostatic sleep pressure and increased slow-wave sleep, while a circadian system sets the thresholds for sleep pressure that correspond to sleep or wakefulness. [10] (Shafer and Kenee 2021)

- Under light/dark cycles, Drosophila displays a bimodal pattern of locomotor activity with a rather narrow burst of activity commencing just before dawn and a larger bout of activity that commences several hours before dusk. Daytime sleep is characterized by shorter, more frequent sleep bouts compared with nighttime sleep68 and arousal thresholds are higher for nighttime sleep19. Thus, nighttime sleep appears to be significantly deeper than daytime sleep in flies. [10] (Shafer and Kenee 2021)

- Approximately 150 neurons support the daily cycling of a core set of circadian clock genes. [10] (Shafer and Kenee 2021)

- neural firing by a given class of clock neuron is expected to have either a sleep- or wake-promoting function, although some studies suggest that the effects of clock neurons on sleep might not be so simple. [10] (Shafer and Kenee 2021)

- One possible explanation for these conflicting results could be that the effects of a specific set of clock neurons on sleep vary across the circadian cycle. Indeed, recent work on the DN1ps revealed

that these neurons govern sleep quality not through a simple increase or decrease of activity, but rather by changes in their patterns of firing. Thus, a clock neuron firing in a particular mode might shift the balance toward sleep, whereas a distinct mode of firing by the same neuron might shift the balance toward wakefulness. [10] (Shafer and Kenee 2021)

- As in humans, the fly's circadian system maintains tight control over sleep even in the presence of significant sleep deprivation: flies that are allowed recovery sleep after deprivation maintain clock-timed decreases in sleep before dawn and dusk and display normal timing and amounts of nighttime sleep. Thus, the circadian clock network likely provides modulatory input into networks mediating homeostatic sleep drive. Indeed, several recent studies have converged on a discrete target of the clock neuron network: ring neurons of the ellipsoid body, a neuronal locus of sleep homeostasis in the central complex. [10] (Shafer and Kenee 2021)

- Four independent groups recently identified neural pathways linking specific cell types within the circadian clock network to the ellipsoid bodies of the central complex. [10] (Shafer and Kenee 2021)

- For example, the DN1p-to-AOTU connection appeared to be a wake-promoting pathway in one study102 and a sleep-promoting pathway in the other103. Notwithstanding the contrasting conclusions of these studies about the roles of different clock output neurons in regulating sleep, their consensus regarding the key roles of the ring neurons is powerful evidence that sleep and circadian control converge in the ellipsoid body. [10] (Shafer and Kenee 2021)

- The two-process model of sleep clearly does not hold when an animal faces acute physiological challenges or fleeting opportunities to mate. Sleep is strongly regulated by environmental factors, including the presence of conspecifics and food availability. [10] (Shafer and Kenee 2021)

- Sleep represents a trade-off with time spent consuming or locating food. Starved flies, like starved rodents, suppress sleep and become hyperactive. [10] (Shafer and Kenee 2021)

- Sleep is also potently modulated by social experience. Flies reared in isolation sleep less during the daytime, revealing long-term plasticity in sleep modulation that is dependent on canonical memory pathways. [10] (Shafer and Kenee 2021)

- Finally, sleep can directly impact social behaviors. For example, sleep loss results in reduced aggression and courtship in flies. [10] (Shafer and Kenee 2021)

- the study of sleep in flies has great potential to shed light on the fundamental biology of sleep and emerging problems related to the role of sleep in human health and disease. [10] (Shafer and Kenee 2021)

- Drosophila will continue to be an important model system for understanding the relationships between the environment, the brain, and sleep. [10] (Shafer and Kenee 2021)

- In the R5 ellipsoid body sleep homeostat, we identified elevated morning expression of activity dependent and presynaptic gene expression as well as the presynaptic protein BRUCHPILOT consistent with regulation by clock circuits. These neurons also display elevated calcium levels in response to sleep loss in the morning, but not the evening consistent with the observed time-dependent sleep rebound. [1] (Andreani et al 2022)

- The circadian process, via phased activity changes in central pacemaker neurons, times and consolidates sleep-wake (Patke et al., 2020). The less well-understood homeostatic process, often assayed after extended sleep deprivation, promotes sleep length, depth, and amount as a function of the duration and intensity of prior waking experience (Deboer and Tobler, 2000; Franken et al., 1991; Huber et al., 2004; Werth et al., 1996). Sleep homeostasis is thought to be mediated by the accumulation of various wake-dependent factors, such as synaptic strength (Tononi and Cirelli, 2014), which are subsequently dissipated with sleep. [1] (Andreani et al 2022)

- While homeostatic drive persists in the absence of a functioning circadian clock (Tobler et al., 1983), homeostatic drive can be modulated by the circadian clock. [1] (Andreani et al 2022)

- Yet the molecular and circuit mechanisms by which the circadian clock modulates sleep homeostasis remain unclear. [1] (Andreani et al 2022)

- Drosophila, a well-established model for investigating the molecular and neural basis of circadian rhythms and sleep. [1] (Andreani et al 2022)

- Sleep is characterized by quiescence, increased arousal thresholds, changes in neuronal activity, and circadian and homeostatic regulation (Campbell and Tobler, 1984). Flies display each of these hallmarks (Hendricks et al., 2000; Shaw et al., 2000; van Alphen et al., 2013) and have simple, well-characterized circadian and sleep neural networks (Dubowy and Sehgal, 2017; Shafer and Keene, 2021). [1] (Andreani et al 2022)

- About 150 central pacemaker neurons that express molecular clocks (Dubowy and Sehgal, 2017). Of these, four small ventral lateral neurons (sLNvs) (per hemisphere) that express pigment dispersing factor (PDF) are necessary for driving morning activity in anticipation of lights on and exhibit peak levels of calcium around dawn (ZT0) (Grima et al., 2004; Liang et al., 2019; Liang et al., 2017; Stoleru et al., 2004). The dorsal lateral neurons (LNds) and a 5th PDF- sLNv are necessary for evening anticipation of lights off and show a corresponding evening calcium peak (ZT8ZT10) (Gossan et al., 2014; Grima et al., 2004; Liang et al., 2019; Liang et al., 2017; Stoleru et al., 2004). The posterior DN1 (DN1ps) consist of glutamate-positive (Glu+) subsets necessary for morning anticipation and Glu- necessary for evening anticipation under low light conditions (Chatterjee et al., 2018). Lateral posterior neurons (LPN) are not necessary for anticipation but are uniquely sensitive to temperature cycling (Miyasako et al., 2007). Specific pacemaker subsets have been linked to wake promotion (PDF+ large LNv (Chung et al., 2009; Parisky et al., 2008; Sheeba et al., 2008), diuretic hormone 31 (DH31+) DN1ps [Kunst et al., 2014]) and sleep promotion (Glu+ DN1ps (Guo et al., 2016), Allostatin A+ LPNs [Ni et al., 2019]), independently of their clock functions. How these neurons regulate homeostatic sleep drive itself remains unsettled. [1] (Andreani et al 2022)

- The sLNvs and LNds appear to communicate to R5 EB neurons through an intermediate set of dopaminergic PPM3 neurons based largely on correlated calcium oscillations (Liang et al., 2019) [1] (Andreani et al 2022)

- The anterior projecting subset of DN1ps provide sleep promoting input to other EB neurons (R2/R4M) via tubercular bulbar (TuBu) interneurons (Guo et al., 2018; Lamaze et al., 2018). Activation of a subset of these TuBu neurons synchronizes the activity of the R5 neurons which is important for sleep maintenance (Raccuglia et al., 2019) [1] (Andreani et al 2022)

- Critically, the R5 neurons are at the core of sleep homeostasis in Drosophila (Liu et al., 2016). [1] (Andreani et al 2022)

- Extended sleep deprivation (12–24 hr) elevates calcium, the critical presynaptic protein BRUCHPILOT (BRP), and action potential firing rates in R5 neurons. The changes in BRP in this region not only reflect increased sleep drive following SD but also knockdown (KD) of brp in R5 decreases rebound (Huang et al., 2020) suggesting it functions directly in regulating sleep homeostasis. [1] (Andreani et al 2022)

- R5 neurons stimulate downstream neurons in the dorsal fan-shaped body (dFB), which are suffi-

cient to produce sleep (Donlea et al., 2014; Donlea et al., 2011; Liu et al., 2016). [1] (Andreani et al 2022)

- Yet how the activity of key clock neurons are integrated with signals from the R5 homeostat to determine sleep drive remains unclear. [1] (Andreani et al 2022)
- Moreover, homeostatic R5 EB neurons integrate circadian timing and homeostatic drive; we demonstrate that activity dependent and presynaptic gene expression, BRP expression, neuronal output, and wake sensitive calcium levels are all elevated in the morning compared to the evening, providing an underlying mechanism for clock programming of time-of-day dependent homeostasis. [1] (Andreani et al 2022)
- After sleep deprivation, flies display a robust sleep rebound throughout the 4.5 hr rebound period in the morning while evening rebound is suppressed (Figure 2a). [1] (Andreani et al 2022)
- we determined if these effects persist under constant darkness (DD). We observed elevated rebound in the morning (CT2.5) relative to the evening (CT10.5), indicating that these differences are not dependent on light (Figure 2c). Altogether, these data suggest that homeostatic rebound sleep is strongly modulated by the internal clock. [1] (Andreani et al 2022)
- TuBu neurons had no effect on rebound [1] (Andreani et al 2022)
- Blocking R5 synaptic output also reduced rebound in both morning and evening [1] (Andreani et al 2022)
- R5 neurons promote sleep in response to deprivation by activating the sleep promoting dFB (Liu et al., 2016). [1] (Andreani et al 2022)
- We were surprised to find that neither morning nor evening SD had much of an effect on gene expression in the R5 neurons [1] (Andreani et al 2022)
- In stark contrast, comparisons of morning and evening timepoints with or without sleep deprivation produces 46–128 differentially expressed genes. Notably, this time-of-day dependent regulation does not appear to be driven by core clock genes in these neurons. [1] (Andreani et al 2022)
- We also observed significant upregulation of genes involved in ionic transport across the plasma membrane, including para, a voltage-gated sodium channel (Catterall, 2000; Loughney et al., 1989), and CG5890, a predicted potassium channel-interacting protein (KChIP) (Figure 8e and g).

Mammalian KChIPs have been shown to interact with voltage-gated potassium channels, increasing current density and conductance and slowing inactivation (An et al., 2000). Two sodium:potassium/calcium antiporters, CG1090 and Nckx30C, were also upregulated (Figure 8e and g). These antiporters function primarily in calcium homeostasis by using extracellular sodium and intracellular potassium gradients to pump intracellular calcium out of the cell when calcium levels are elevated (Haug-Collet et al., 1999). [1] (Andreani et al 2022)

- Amongst the most significantly upregulated genes in our dataset, we found six genes that were previously identified as activity-regulated genes in Drosophila (ARGs; sr, Cdc7 (also known as l(1)G0148), CG8910, CG14186, CG17778, hr38) [1] (Andreani et al 2022)

- R5 neurons are assembling a greater number of mature active zones for neuronal output. Upregulation of para and the predicted KChIP CG5890, which should increase the voltage-gated conductance of sodium and potassium ions across the membrane, supports the idea that R5 neurons may be primed for greater action potentials in the morning. Upregulation of the two sodium:potassium/calcium antiporters suggests that intracellular calcium levels are elevated in the morning, again consistent with the idea that these neurons are more active in the morning. [1] (Andreani et al 2022)

- SD/extended wake results in the upregulation of many synaptic proteins (Gilestro et al., 2009). Most notable is the presynaptic scaffolding protein BRP, which is important for synaptic release (Matkovic et al., 2013), and is upregulated in the R5 neurons following 12 hr of SD (Liu et al., 2016). KD of brp in R5 neurons decreases rebound response to SD (Huang et al., 2020), suggesting that it is necessary for accumulating and/or communicating homeostatic drive. We hypothesized that differences in the propensity for R5 to induce sleep rebound in the morning/evening may be due to changes in synaptic strength that can be observed by tracking levels of BRP. [1] (Andreani et al 2022)

- The calcium concentration in R5 neurons increases following twelve hours of SD, suggesting that extended wakefulness can induce calcium signaling in these neurons. Blocking the induction of calcium greatly reduces rebound, supporting a critical role for calcium signaling in behavioral output (Liu et al., 2016). Furthermore, R5 neurons display morning and evening cell-dependent peaks in calcium activity across the course of the day indicating that calcium is also modulated by the clock network (Liang et al., 2019) [1] (Andreani et al 2022)

- we observed very little gene expression significantly altered in response to our 2.5 hr sleep depriva-

tion. On the other hand, we did identify elevated expression of activity-dependent and presynaptic genes in the morning independent of sleep deprivation. Consistent with this finding, we also observe elevated levels of the presynaptic protein BRP that is absent in the absence of Clk. These baseline changes are accompanied by an elevated calcium response to sleep deprivation in the morning mirroring the enhanced behavioral rebound in the morning. [1] (Andreani et al 2022)

- We observed significant upregulation of several genes involved in synaptic transmission (Syx1a, Rim, nSyb, unc-104, Srpk79D, para, CG5890) evincing a permissive active state for R5 neurons in the morning. This is accompanied by elevated levels of the key presynaptic protein BRP in the morning compared to evening. [1] (Andreani et al 2022)

- In Drosophila, a crucial component of the machinery for sleep homeostasis is a cluster of neurons innervating the dorsal fan-shaped body (dFB) of the central complex2,3. Artificial activation of these cells induces sleep2, whereas reductions in excitability cause insomnia3,4. [7] (Pimentel et al 2016)

- dFB neurons in sleep-deprived flies tend to be electrically active, with high input resistances and long membrane time constants, while neurons in rested flies tend to be electrically silent3. [7] (Pimentel et al 2016)

- Here we demonstrate state switching by dFB neurons, identify dopamine as a neuromodulator that operates the switch, and delineate the switching mechanism. Arousing dopamine4–8 caused transient hyperpolarization of dFB neurons within tens of milliseconds and lasting excitability suppression within minutes. Both effects were transduced by Dop1R2 receptors and mediated by potassium conductances. The switch to electrical silence involved the downregulation of voltage-gated A-type currents carried by Shaker and Shab, and the upregulation of voltage-independent leak currents through a two-pore-domain potassium channel that we term Sandman. Sandman is encoded by the CG8713 gene and translocates to the plasma membrane in response to dopamine. dFB-restricted interference with the expression of Shaker or Sandman decreased or increased sleep, respectively, by slowing the repetitive discharge of dFB neurons in the ON state or blocking their entry into the OFF state. [7] (Pimentel et al 2016)

- Illumination at 630 nm, sustained for 1.5 s to release a bolus of dopamine (Extended Data Fig. 1), effectively stimulated locomotion (32/38 trials; Fig. 1a, b). dFB neurons paused in successful (but not in unsuccessful) trials (Fig. 1a, b), and their membrane potentials dipped by 2–13 mV (7.50 ±

0.56 mV; mean ± standard error of the mean (s.e.m.)) below the baseline during tonic activity. [7] (Pimentel et al 2016)

- The tight correlation between the suppression of dFB neuron spiking and the initiation of movement [7] (Pimentel et al 2016)
- Flies with enhanced dopaminergic transmission exhibit a short-sleeping phenotype that requires the presence of a D1-like receptor in dFB neurons [7] (Pimentel et al 2016)
- Loss of Dop1R2 increased sleep during the day and the late hours of the night, by prolonging sleep bouts without affecting their frequency [7] (Pimentel et al 2016)
- Like optogenetically stimulated secretion, focal application of dopamine hyperpolarized the cells and suppressed their spiking [7] (Pimentel et al 2016)
- While a single pulse of dopamine transiently hyperpolarized dFB neurons and inhibited their spiking, prolonged dopamine applications switched the cells from electrical excitability (ON) to quiescence (OFF) [7] (Pimentel et al 2016)
- dFB neurons in the ON state expressed two types of potassium current: voltage-dependent A-type16 and voltage-independent non-A-type currents. d Extended Data Fig. 6a-c). The current–voltage (I-V) relation of IA resembled that of Shaker, the prototypical A-type channel. Non-A-type currents showed weak outward rectification with a reversal potential of -80 mV (Fig. 3e, g), consistent with potassium as the permeant ion, and no inactivation [7] (Pimentel et al 2016)
- Switching the neurons OFF changed both types of potassium current. IA diminished by one-third (Fig. 3e, f), whereas Inon-A nearly quadrupled when quantified between resting potential and spike threshold. dFB neurons thus upregulate IA in the sleep-promoting ON state (Fig. 3e, f). When dopamine switches the cells OFF, voltage-dependent currents are attenuated and leak currents augmented depletion of voltage-gated A-type (KV) channels (which predominate in the ON state) should tip the cells towards the OFF state; conversely, loss of leak channels (which predominate in the OFF state) should favour the ON state. [7] (Pimentel et al 2016)
- small LNvs (sLNvs) are bursting neurons, and Ih is necessary to achieve the high-frequency bursting firing pattern characteristic of both types of LNvs in females. [5] (Fernandez-Chiappe et al 2021)

- Circadian (circa: around, diem: day) rhythms are biological rhythms with a period of ;24 h that have evolved in essentially all organisms. [5] (Fernandez-Chiappe et al 2021)

- The large LNvs (lLNvs), on the other hand, are highly relevant for arousal and the PDF they release provides wake promoting functions (Parisky et al., 2008; Shang et al., 2008; Sheeba et al., 2008a). [5] (Fernandez-Chiappe et al 2021)

- we demonstrate that perturbing Ih causes a decrease in the frequency of LNvs bursting that is accompanied by a reduction in PDF immunoreactivity and in the complexity of sLNv axonal termini. [5] (Fernandez-Chiappe et al 2021)

- We report that dFB neurons communicate via inhibitory transmitters, including allatostatin-A (AstA), with interneurons connecting the superior arch with the ellipsoid body of the central complex. These "helicon cells" express the galanin receptor homolog AstA-R1, respond to visual input, gate locomotion, and are inhibited by AstA, suggesting that dFB neurons promote rest by suppressing visually guided movement. [2] (Donlea et al 2018)

- Helicon cells provide excitation to R2 neurons of the ellipsoid body, whose activity-dependent plasticity signals rising sleep pressure to the dFB. [2] (Donlea et al 2018)

- The behavioral hallmarks of sleep are manifold. They include inactivity, reduced responsiveness to external stimuli, rapid reversibility, and homeostatic rebound after sleep loss. Any sleep control system must therefore fulfill a multitude of functionsblocking locomotor activity, gating sensory pathways, inhibiting arousal systems, relieving sleep pressure—and perhaps also directly influence processes germane to a fundamental purpose of sleep, be it metabolic recovery (Vyazovskiy and Harris, 2013; Walker et al., 1979), memory consolidation (Wilson and McNaughton, 1994), or synaptic scaling (Tononi and Cirelli, 2003). [2] (Donlea et al 2018)

- two dozen neurons (of a total of 100,000 in the brain) suffices to induce sleep in Drosophila (Donlea et al., 2011). [2] (Donlea et al 2018)

- We find that dFB neurons induce sleep via a range of inhibitory transmitters that include the neuropeptide allatostatin-A (AstA). Among the targets of AstA are a group of interneurons of the central complex that we term helicon cells. These neurons are inhibited by sleep-promoting AstA, excited by visual input, permissive for locomotion, and presynaptic to R2 ring neurons of the ellipsoid body, whose activity has been linked to the accumulation of sleep debt (Liu et al., 2016).

dFB-mediated inhibition of helicon cells may thus account for three cardinal features of sleep: elevated visual thresholds, immobility, and the dissipation of sleep need. [2] (Donlea et al 2018)

- A Sleep-Promoting Signal from dFB Neurons [2] (Donlea et al 2018)
- Helicon Cells: Targets of dFB Neurons with Projections to the Ellipsoid Body [2] (Donlea et al 2018)
- dFB Neurons Inhibit Helicon Cells and Their Visual Responses [2] (Donlea et al 2018)
- helicon cells (Figure 4A) were found in one of two states: a DOWN state characterized by the near absence of spikes (firing rate ¡ 1 Hz) and an UP state in which the neurons fired persistently, with occasionally metronomic precision, at rates of 16.9 ± 3.6 Hz (Figures 4B and 4C). An average voltage difference of 10.9 ± 2.3 mV (mean ± SEM, n = 10 cells) separated the membrane potential baselines of the two states. [2] (Donlea et al 2018)
- Spontaneous movements were initiated with approximately 4-fold higher probability when the recorded cell was in the UP rather than in the DOWN state (Figure 4E). Together, these results suggest that helicon cells play a permissive role in visually guided movement. [2] (Donlea et al 2018)
- Helicon Cells Gate Locomotion [2] (Donlea et al 2018)
- Helicon Cells Excite R2 Ring Neurons [2] (Donlea et al 2018)
- Helicon Cell Activation Induces Rebound Sleep [2] (Donlea et al 2018)
- These results demonstrate a sleep-promoting effect of inhibiting helicon cells, but they also suggest that helicon cells are only one of several dFB outputs used to induce sleep. [2] (Donlea et al 2018)
- The contours of an autoregulatory loop have thus emerged in which sleep-promoting dFB neurons communicate via helicon cells with R2 neurons, and the activity of these ring neurons is relayed back to dFB neurons (Figure 8). We imagine that, as sleep pressure builds during prolonged R2 neuron firing, activity-dependent plasticity (Liu et al., 2016) augments the excitatory drive to dFB neurons or instructs them to step up their intrinsic excitability. As a result, dFB neurons switch to the electrically active state and release inhibition. This pushes helicon cells into the hyperpolarized DOWN state (Figure 4), mutes their spiking, and deprives R2 neurons of a powerful source of excitation [2] (Donlea et al 2018)

- They include where precisely along the still unexplored R2dFB neuron interface sleep debt accrues, in what physical form it is stored, how its accumulation to threshold actuates the dFB switch, and how the accumulated sleep debt is cleared. [2] (Donlea et al 2018)

- We find that the power of these slowwave oscillations increases with sleep need and is subject to diurnal variation. [8] (Raccuglia et al 2019)

- Optical multi-unit voltage recordings reveal that single R5 neurons get synchronized by activating circadian input pathways. [8] (Raccuglia et al 2019)

- (R5) We show that this synchronization depends on NMDA receptor (NMDAR) coincidence detector function, and that an interplay of cholinergic and glutamatergic inputs regulates oscillatory frequency. Genetically targeting the coincidence detector function of NMDARs in R5, and thus the uncovered mechanism underlying synchronization, abolished network-specific compound slow-wave oscillations. It also disrupted sleep and facilitated light-induced wakening, establishing a role for slow-wave oscillations in regulating sleep and sensory gating. [8] (Raccuglia et al 2019)

- However, how specific neural networks contribute to generating compound oscillations and whether these oscillations represent a functional unit for sleep regulation largely remains unclear. [8] (Raccuglia et al 2019)

- However, in invertebrates, it is unknown whether electrical oscillations can gate specific behaviors and whether an electrophysiological sleep correlate, such as slow-wave oscillations, exists or is involved in sleep regulation. Local field potential (LFP) measurements in the Drosophila brain indicate that the frequency of large-scale compound neuronal activity is reduced during sleep [8-10], opening up the possibility that, comparable to vertebrates, slow oscillatory activity could be involved in mediating sleep. [8] (Raccuglia et al 2019)

- Here, we discover sleep-regulating, network-specific delta oscillations within the R5 [14-16] network of the Drosophila ellipsoid body, which is situated at a crossroad involved in sleep regulation [14, 17, 18] and sensory processing [15-20] [8] (Raccuglia et al 2019)

- (R5) Disrupting this synchronization and thus the emergence of compound delta oscillations affects the flies sleep patterns and alters sensory gating during sleep. [8] (Raccuglia et al 2019)

- Our work suggests that slow-wave oscillations and sleep could be fundamentally interconnected across phyla. Slow-wave oscillations may therefore potentially represent an evolutionarily con-

served strategy for network mechanisms regulating internal states and sleep. [8] (Raccuglia et al 2019)

- We targeted expression of the GEVI ArcLight specifically to R5 (also sometimes referred to as R2) [14] neurons in the Drosophila brain. This defined network of 10–12 cells per hemisphere (Figure 1A) projects to the ellipsoid body and is involved in sleep regulation [14, 17, 18]. [8] (Raccuglia et al 2019)

- In vivo recordings of the dendritic processes of R5 neurons (dorsal bulb; Figure 1A) identified electrical compound activity. Power analyses showed clear peaks around 1 Hz. [8] (Raccuglia et al 2019)

- Although, at early hours (ZT 0–3), no peak in the power spectrum was detected, delta oscillations around 1 Hz (integrated delta power at 0.5–1.5 Hz) became apparent during later hours of the day (ZT 8–12; Figures 1C–1E). Delta power peaked between ZT 13 and 16, which overlaps with the ZT that we measured as the animals mean onset of ”consolidated” sleep [8] (Raccuglia et al 2019)

- Strikingly, delta power was increased after increasing the animals' sleep pressure through sleep deprivation. [8] (Raccuglia et al 2019)

- Together, our data demonstrate that the power of delta oscillations is correlated to the animal's behavioral onset of sleep while being subject to diurnal variation and homeostatic sleep regulation. [8] (Raccuglia et al 2019)

- Individual R5 neurons showed oscillatory activity with peak frequencies similar to the compound signal. Temporal correlation analysis between electrical patterns of simultaneously recorded R5 neurons showed that most depolarization phases occurred with a time lag of ¡50 ms (median = 13 ms; Figure 2G). Temporal overlap of single-unit activity could therefore be at the basis of the observed compound oscillations. [8] (Raccuglia et al 2019)

- We found that single-unit delta power was reduced in the morning. Moreover, the correlation between electrical patterns of single units was increased later in the day. [8] (Raccuglia et al 2019)

- NMDARs are coincidence detectors, and activation requires simultaneous ligand binding and membrane depolarization to remove Mg2+ ions blocking the channel pore. [8] (Raccuglia et al 2019)

- single-unit delta-band oscillations require network activity potentially generated by NMDAR-mediated signaling. Interestingly, sleep deprivation leads to an upregulation of NMDAR transcripts in R5 neurons. [8] (Raccuglia et al 2019)

- TuBu neurons convey sensory and circadian information, and functional connectivity has been demonstrated for subsets of ellipsoid body ring neurons. [8] (Raccuglia et al 2019)

- Activation of the TuBu neurons reinstated delta oscillations at high [Mg2+]e, and single units showed a reversible increase in delta power (Figures 3A and 3B). [8] (Raccuglia et al 2019)

- (TuBu activation) Importantly, we also observed a reduction of the time lag and an increased correlation of up-states between individual units (Figure 3C). This demonstrates that activity transmitted via TuBu neurons can synchronize R5 neurons. [8] (Raccuglia et al 2019)

- No delta activity was detected within the TuBu neurons (neither at the presynaptic compound level nor at the level of individual cell bodies; Figures S4A-S4D), suggesting that oscillations could be generated at the level of R5 neurons. [8] (Raccuglia et al 2019)

- removing NMDARs "from the equation" by applying APV completely prevented activation of R5 units (Figure S3C). Thus, NMDARs are required for oscillatory activity per se, and [Mg2+]e levels (and therefore likely NMDAR coincidence detection) were decisive as to whether the network stimulation would increase either single-unit oscillatory frequency or lead to multi-unit synchrony. [8] (Raccuglia et al 2019)

- acetylcholine can provide the coincident signal to "unblock" NMDARs required for synchronization of single units at the basis of delta-band oscillations. [8] (Raccuglia et al 2019)

- Our results indicate that NMDARs and, more specifically, the NMDAR Mg2+ block are crucially involved in generating R5-specific compound oscillations [8] (Raccuglia et al 2019)

- In vivo whole-cell patch-clamp recordings of single R5 neurons of control animals expressing non-mutated NMDAR subunit 1 showed rhythmic bursting at ZT 8–12, and power spectral analysis showed a clear peak around 1 Hz (Figures 5A–5C). This peak was not detected when analyzing recordings from animals expressing NMDARMg-/- in R5. [8] (Raccuglia et al 2019)

- ursts were unaltered (Figures 5D and 5E). Strikingly, in flies expressing NMDARMg-/-, inter-burst intervals did not follow regular patterns [8] (Raccuglia et al 2019)

- flies that expressed NMDARMg-/- in R5 neurons. Flies slept significantly less in total compared to controls, , and the number of sleep episodes was increased (Figure 6D) while sleep episode duration was decreased. Thus, flies no longer capable of multi-unit synchronization in R5 neurons woke up more frequently, and it took them longer to fall asleep. [8] (Raccuglia et al 2019)
- Expressing NMDARMg-/- in R5 significantly reduced rebound sleep after sleep deprivation [8] (Raccuglia et al 2019)
- Expressing NMDARMg-/-: the threshold for wakening was lower in the mutant and a significantly larger fraction of flies was wakened [8] (Raccuglia et al 2019)
- the mechanisms underlying compound delta oscillations in the R5 network not only regulate sleep drive but also the gating of stimulus-triggered wakening, potentially following an evolutionarily conserved strategy. [8] (Raccuglia et al 2019)
- In vertebrates, sleep and sleepiness are thought to be tightly interlinked with the synchronization of neuronal activity, resulting in increased compound slow-wave oscillations [2, 3] [8] (Raccuglia et al 2019)
- Our data suggest that compound delta oscillations specific to the sleep-regulating R5 network are generated by circadian drive transduced via TuBu neurons. We show that optogenetic activation of TuBu neurons increases single-unit power and synchronizes R5 neurons (Figure 3), which should result in an increase of compound delta power (Figure 1) and thus internal sleep drive. This is consistent with thermogenetic activation of TuBu neurons increasing the total amount of sleep in flies [15]. High levels of TuBu neuron output could be generated by altering activity in sleep-modulating DN1 circadian clock neurons [32], which form direct connections with the TuBu neurons [17]. [8] (Raccuglia et al 2019)
- The R5 network also receives excitatory input from Helicon cells [18], a potential source of cholinergic input. We here provide evidence that nAChRs act as prime candidates to provide concurrent depolarization required for NMDAR coincidence detection in R5 neurons. [8] (Raccuglia et al 2019)
- Helicon cells also receive visual information and are part of a recurrent circuit mediating homeostatic sleep pressure regulation [18, 35]. Thus, R5 oscillatory activity is likely regulated via a complex interplay of sensory input [20], circadian rhythms [15, 17], and homeostatic sleep pressure regulation. [8] (Raccuglia et al 2019)

- Expression levels of NMDARs in R5 neurons have previously been associated with the regulation of sleep drive [14]. [8] (Raccuglia et al 2019)

- Our data suggest that NMDAR coincidence detection gates neuronal synchronization of delta-wave activity within the R5 network to increase the power of sleep-relevant compound oscillations (Figures 3 and 5). Indeed, at the single-cell level, expressing Mg2+ block-deficient NMDARs in R5 neurons led to irregular activity patterns (Figure 5), which could be at the basis of impaired synchronization and disrupted compound oscillations. [8] (Raccuglia et al 2019)

- oscillatory activity in Drosophila R5 neurons is reminiscent of up- and down-states occurring at the level of mammalian cortical networks during deep sleep [36]. We thus hypothesize that the oscillations observed here are comparable to sleep-regulating thalamocortical oscillations [37-39] as well as network-specific oscillations observed during sleep deprivation in vertebrates (local sleep) [2, 40, 41]. Thus, the R5 network could be functionally analogous to the thalamus, as network-specific synchronization of slowwave activity within the thalamus plays a crucial role in maintaining sleep [37-39] and sensory gating [42]. [8] (Raccuglia et al 2019)

- whether cell-autonomous conductances contribute to sustained rhythmic activities of single R5 neuron remains an open question. [8] (Raccuglia et al 2019)

- Here, we describe a neural mechanism in Drosophila that creates neural filters that engender a brain state allowing for quiescent behavior by generating coherent slow-wave activity (SWA) between sleep-need- (R5)4 and locomotion-promoting neural networks. [9] (Manuscript)

- coherent oscillations provide the mechanistic basis for a neural filter by temporally associating opposing signals resulting in reduced functional connectivity between locomotion-gating and navigational networks. We propose that the temporal pattern of SWA provides the structure to create a ”breakable” filter, permitting the animal to enter a quiescent state, while providing the architecture for strong or salient stimuli to ”break” the neural interaction, consequently allowing the animal to react. [9] (Manuscript)

- We recently identified that electrical SWA (0.5- 1.5 Hz) in the R5 network of the Drosophila central complex is tied to undisrupted sleep, indicating that SWA is also a network-autonomous marker of sleep need across phyla [9] (Manuscript)

- Apart from the R5 network (with network here referring to interconnected R5 neurons with any in- and output connections) the Drosophila central complex comprises two additional prominent

networks: that of the dorsal fan-shaped body (dFSB) and that of locomotion-promoting and visual signal-transducing helicon cells5. [9] (Manuscript)

- We found that network activity of R5 and the dFSB showed increased synchronization at night compared to the day. we observed changes of electrical activity patterns in R5 and the dFSB between night and day, with an increase in power at night and also in the morning following sleep deprivation. (The plot shows only dFSB...) [9] (Manuscript)
- Indeed, demonstrating that neither sensory stimuli nor VNC activity shaped dFSB SWA, we observed coherent nocturnal SWA ex vivo. Moreover, simultaneously acquired recordings of single dFSB neurons revealed low overall activity in the morning, while SWA markedly increased at night. However, activity was only loosely correlated between single cells4 regardless of whether measured in the morning or at night, unlike what we previously observed for the R5 network. Therefore, changes in single-cell excitability of dFSB neurons might be the underlying source of compound oscillations, rather than the synchronization of single units. [9] (Manuscript)
- Optogenetic stimulation of R5 reliably induced or amplified dFSB presynaptic SWA following the end of our activation protocol (Extended Data Fig. 2a-c,g), indicating that synaptic output of R5 suffices to induce ongoing SWA. [9] (Manuscript)
- The R5 stimulation-induced increase in power of the observed SWA in dFSB neurons was most pronounced at nighttime (Extended Data Fig. 2c), demonstrating the influence of the time of day on interactions between these neural circuits. [9] (Manuscript)
- Optogenetic activation of the dFSB elicited pronounced SWA in R5 during stimulation at nighttime but none during the day. In a minority of cases, R5 SWA persisted even after optogenetic activation ceased (Extended Data Fig. 2i), suggesting that increased synaptic output as a result of increased activity of the dFSB at night can, in principle, entrain R5 oscillations and might thus have an effect on a longer time scale. [9] (Manuscript)
- optogenetically silencing the dFSB reduced oscillatory power of the R5 network (Extended Data Fig. 2k,l), further supporting the notion that increased activity within the neurons of the dFSB facilitates SWA in R5. [9] (Manuscript)
- Sometimes R5 SWA partially recovered after the start of optogenetic inhibition (Extended Data Fig. 2l), suggesting that other inputs to R5 or cell-autonomous processes stabilize R5 SWA at night. [9] (Manuscript)

- prominent structures of the central complex exhibit similar, but not identical mutually interactive oscillations at night [9] (Manuscript)
- At night, the dFSB increases activity to generate compound oscillations (Extended Data Fig. 1c). Importantly, these in turn switch on coherent oscillatory activity in the R5 network (Fig. 1). [9] (Manuscript)
- In accordance with the locomotion of flies in this arena being subject to their sleep need, the mean velocity of sleep- deprived flies was significantly reduced compared to rested flies. [9] (Manuscript)
- To mimic SWA in our behavioral assay, we stimulated R5 optogenetically at 1 Hz. We found that overall optogenetic stimulation of R5 reduced locomotor activity but flies still displayed short walking bouts as well as frequent grooming. Locomotion recovered within minutes after stimulation offset. This is in line with R5 activity entraining the involved neural networks to facilitate flies sleep4 by potentially filtering out sensory stimuli. [9] (Manuscript)
- Demonstrating that they could react to strong stimuli per se, flies reacted to an air puff during 1 Hz R5 optogenetic stimulation by increasing their walking velocity. These findings suggest that R5 activation at 1 Hz reduces locomotor activity while leaving the ability to walk and respond to strong stimuli. [9] (Manuscript)
- We therefore conclude that 1 Hz R5 activation induces a behavioral state that we refer to as ”quiescent”, which is characterized by reduced locomotor activity, but also by periods of prolonged rest (that may include sleep, Extended Data Fig. 3k,l) and also grooming (see Supplemental Video 1). [9] (Manuscript)
- Of note, stimulating R5 neurons at 0.1 Hz did not lead to a measurable impact on the flies' behavior (Fig. 2i and Extended Data Fig. 3h). Therefore, the frequency or intensity of R5 activation mattered for inducing quiescence. [9] (Manuscript)
- our analysis revealed that R5 neurons are highly interconnected with the helicon network that responds to visual stimuli. In addition, R5 neurons are one of the main pre- and postsynaptic partners of helicon cells. [9] (Manuscript)
- Consistent with previous observations showing that dFSB neurons inhibit visually evoked responses in helicon cells5, we found that helicon cells are also connected to the dFSB [9] (Manuscript)

- Simultaneous in vivo recordings of helicon and R5 at their overlapping presynaptic sites confirmed that in both R5 and helicon networks SWA were observable, particularly at night. [9] (Manuscript)
- We observed two states (Fig. 3c,d and Extended Data Fig. 5b-d): a synchronized state and a "shifted" state in which the electrical patterns of R5 were correlated with helicon activity, but preceding R5 activity by 50 to 200 ms [9] (Manuscript)
- Turning back to ex vivo recordings, we only observed the synchronized state (Fig. 3g,h and Extended Data Fig. 5g), indicating that the shifted state could result from interferences with sensory input. [9] (Manuscript)
- we first performed in vivo dual color voltage recordings during the midday. Correlation coefficients of R5 and helicon SWA were comparable to those of morning recordings. On the contrary, sleep deprivation and, therefore, high homeostatic sleep pressure, was sufficient to increase the correlation between R5 and helicon SWA (Fig. 3e) in the morning, without increasing oscillatory power in helicon (Extended Data Fig. 5c), which opens the possibility of homeostatic influence. [9] (Manuscript)
- Strikingly, isolated homeostatic sleep drive (sleep depriving flies at night and activity measured in the following morning hours), led to animals only displaying the shifted and not the synchronized state (Extended Data Fig. 5b). Therefore, strong homeostatic drive appears to suffice for networks to enter the shifted state, but not to create the synchronized state. (Were the neurons bursting in the shifted state?) [9] (Manuscript)
- In the absence of light, SWA profiles still showed an increased correlation at nighttime compared to morning hours (Fig. 3e), suggesting that, along with homeostatic, circadian drives could contribute to creating coherence between the networks' electrical patterns. [9] (Manuscript)
- Flies with a disrupted circadian clock that were kept in darkness no longer showed a statistically significant "nocturnal" increase in network coherence (Fig. 3e). Strikingly, however, control flies kept in darkness nearly exclusively showed synchronized profiles. As this differs from flies subject to light during the day (58.54synchronized, n=41), this could be in line with an absence of sensory input facilitating the synchronized state. [9] (Manuscript)
- Taken together, the circadian clock and the absence of sensory input generate a nocturnal increase in network synchrony that could represent a closed filter state while visual input facilitates a shifted state that could represent open filter settings during quiescence. [9] (Manuscript)

- However, as inhibitory pulse-coupled oscillators are known to usually stay out of sync27, we found that in order to simulate synchronization patterns similar to those experimentally observed, we required mixed excitatory and inhibitory output of R5 neurons. [9] (Manuscript)

- In the model, we varied the relative strength of the inhibitory and excitatory synaptic coupling among R5 neurons and observed that synchronization correlated with the strength of excitation compared to inhibition within the network [9] (Manuscript)

- Strikingly, we identified several cholinergic R5 sites (Extended Data Fig. 6b), supporting an R5 network with mixed inhibitory and excitatory output as source for the observed oscillations. [9] (Manuscript)

- Helicon, as a purely excitatory recurrent network that is also connected to R5, was modeled using neurons that switch from a more depolarized and active state in the morning (”upstate”) to a more hyperpolarized, and less active state (”downstate”) at nighttime (Fig. 4a). Indeed, providing experimental support for our model, we found that single helicon cells were significantly more hyperpolarized at night compared to the morning (Extended Data Fig. 6d). Our simulation is in line with hyperpolarizing signals, potentially mediated by the dFSB5 and/or other sources, paving the path for R5-mediated entrainment of helicon activity at night (Fig. 4b,c) by rendering helicon less responsive to inhibitory (while still responsive to excitatory) input from R5 when helicons resting membrane potential is closer to the chloride reversal potential32. [9] (Manuscript)

- To further experimentally validate our model, we performed ex vivo helicon imaging experiments, while briefly optogenetically stimulating R5 at 1 Hz for 20 seconds during the day. We observed that even at this short time scale, transient, and sometimes persistent, entrainment worked per se (Extended Data Fig. 7g-i). Because R5 activation can also entrain dFSB activity during the day (Extended Data Fig. 2a-c), we suspect that this interaction would effectively set helicon cells to the downstate (night setting), allowing for entrainment of helicon by R5. Indeed, this is also in line with our behavior experiments, where, when stimulating R5 for a significantly longer period, we were able to induce behavioral quiescence that could even endure post stimulation (Fig. 2d-g). [9] (Manuscript)

- ”shifted state”. We reasoned that a main difference between ex vivo and in vivo recordings would be the absence of sensory input or motor feedback (compare Fig. 3e, flies in DD). To simulate this, we applied additional input to helicon (see Supplementary information for details). Indeed,

this additional input shifted the activity peaks of helicon and R5 networks by approximately 100 ms (Extended Data Fig. 7f), closely resembling the experimentally observed ”shifted state” (Fig. 3c,d and Extended Data Fig. 5b). This is also in line with our experimental data, which show that the balance controls the degree of synchronization between excitatory and inhibitory drive and determines whether the networks are in the shifted or synchronized configuration. [9] (Manuscript)

- While all genotypes showed slight arousal to red light, helicon stimulation led to strong arousal, whilst arousal during R5 stimulation was comparable to the control [9] (Manuscript)

- Interestingly, compared to R5 activation alone, synchronous activation of R5 and helicon led to a somewhat stronger reduction of locomotor activity (Fig. 4g,h) and only a small fraction of flies was awakened by green light (Extended Data Fig. 8a-c). Indeed, activating R5 and helicon simultaneously even blocked air puff responses (Extended Data Fig. 8d-g), suggesting that R5 and helicon synchronization can establish a strong neural filter that might also extend to other modalities [9] (Manuscript)

- Together, these findings indicate that sleep drive mediated by R5 oscillations overrides gating of locomotion by helicon cells and further demonstrates that synchronization between these networks locks the locomotion-initiating networks into a state that, dependent on sleep need, reduces sensory processing and behavioral responsiveness. [9] (Manuscript)

- Our connectome analysis revealed that helicon cells as well as R5 neurons are connected to downstream EPG neurons (Fig. 5a), which represent the flys heading direction and initiate body turns towards or away from sensory stimuli and thus partake in reflecting the external world and controlling navigation [9] (Manuscript)

- (Helicon and R5 antagonistically regulate downstream head direction neurons) Strikingly, we found that optogenetic R5 activation led to net hyperpolarization of EPG neurons, while activation of helicon cells led to strong depolarizations (Fig. 5f-h), indicating that R5 and helicon cells modulate EPG activity via antagonistic neurotransmitter inputs. [9] (Manuscript)

- Depolarizing EPG neurons at 1 Hz, the frequency band of synchronized R5 and helicon, had no effect on locomotion (Extended Data Fig. 9a). In line with strong drive to EPG steering animal behavior away from quiescence, activating a set of EPG neurons at higher frequencies (10 Hz), approximating high excitatory input via helicon, altered locomotor activity (Fig. 5i,j and Extended Data Fig. 9b,c) along with turning behavior (Fig. 5j,k). [9] (Manuscript)

- we directly tested the sensory filtering abilities of R5-based activity. optogenetic stimulation of R5 induces hyperpolarization of EPG in vivo. We next13 widened the illumination to cover the brain and both eyes (Fig. 6b). In control flies, EPG neurons showed robust activation to visual input during the day (Fig. 6a,b). At night, however, visually-evoked depolarization was clearly attenuated. Strikingly, optogenetic activation of R5 during the delivery of the visual input at daytime (Fig. 6c,d) attenuated visually-evoked responses in EPG, reminiscent of night-time recordings and confirming the visual filtering properties of R5. [9] (Manuscript)

- Together, our study uncovers a mechanism in which circadian and homeostatic sleep need create coherent electrical activity across different networks at night (oscillating around 1 Hz, Fig. 1, 3). Facilitated through the dFSB (Fig. 1), the R5 network can overrule input of locomotion-promoting helicon to EPG (Fig. 4), by temporally associating helicon activity (Fig. 3) and thus creating a neural filter (Fig. 6) to promote quiescent behavior (Fig. 2). [9] (Manuscript)

- The occurrence of brain-wide SWA during deep sleep9,10 as well as network-specific SWA during local sleep in awake individuals39,40 suggests that coherent SWA could represent a neural filtering mechanism that regulates sensory processing and behavioral responsiveness [9] (Manuscript)

- We here show a mechanism that creates SWA across networks involved in regulating sleep (e.g. homeostatic sleep regulation), setting up a sensory filter that attenuates visually evoked activity in downstream navigational neurons. [9] (Manuscript)

- Therefore, sensory filtering and homeostatic sleep regulation can closely14 interact, eventually allowing the animal to transfer from quiescent behaviors prior to sleep to sleep. [9] (Manuscript)

- In the morning setting, the observed networks (dFSB-helicon-R5) act independent of each other, allowing gating of locomotion and updating of the head direction system (Fig. 6e). In the night setting, circadian and homeostatic regulation promote the entrainment of synchronized SWA between networks that opposingly regulate behavioral responses to visual stimuli. As a consequence, the functionally antagonistic inputs of neighboring helicon and R5 synapses to the head direction system (EPG)6,7,38 cancel each other out, and set up a filter that reduces functional connectivity within the system to initiate a quiescent state that can allow for sleep need to transition into sleep. [9] (Manuscript)

- SWA, comprised of oscillating up- (depolarized) and down-states (hyperpolarized)9, could follow a general architecture of breakable neural filters by providing a limited time window of transducing

sensory information (during up-states) [9] (Manuscript)

- Importantly, the synchronized state between R5 and helicon was only observed during night- time settings. Therefore, it is the interplay between diurnal/circadian factors and homeostatic regulation that allows for optimal regulation of sensory filtering depending on internal state and time of day. [9] (Manuscript)

- Underlining this multi-layered regulation, artificial stimulation of the dFSB only induced SWA in R5 at night. This places the dFSB as a prime candidate to exert inhibitory drive to helicon5, as a prerequisite for coherence of R5 and helicon activity. [9] (Manuscript)

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- during SWA, our experiments suggest that flies can remain responsive to strong stimuli arguing that the filter can be bypassed or broken. [9] (Manuscript)

- stimulating R5 and helicon in sync (representative of the synchronized configuration) showed to be more effective than stimulating R5 alone (representative of the synchronized or shifted configuration) in filtering out sensory stimuli and even extended the sensory modality of this neural filter, from a visual filter to also filtering mechanosensory input. Importantly, our experimental data and simulation suggest that the shifted state is facilitated by external sensory information. [9] (Manuscript)

- Because SWA is associated with quiescence across phyla, we postulate that the neural interactions discovered here are not restricted to flies but could represent a general neural filtering mechanism43 that suppresses sensory processing to facilitate the shift from a world- driven to an internally-driven brain state [9] (Manuscript)