traits important in resistance to a range of extrinsic environmental hazards. When these genetic correlations are negative, we might expect that condition–environment interactions will accelerate the evolutionary response of aging in the direction of the classic prediction. When positive, condition–environment interactions might act to slow down or even reverse the sign of the classic prediction. Genetic covariance between stress-resistance traits (e.g. resistance to starvation, heat or cold stress) and life span, within populations, generally tends to be positive or absent [16–20], which suggests that increases in extrinsic mortality imposed by environmental stressors might generally promote the evolution of longer life, consistent with the findings by Chen and Maklakov [2]. That said, before we can make conclusions here, we will need to better understand patterns of genetic covariance between susceptibility to predation and infectious disease and intrinsic components of aging, given the pervasiveness of these mortality sources in natural populations. Clearly, the Chen and Maklakov study [2] provides a new insight into the evolution of life span. Ironically, in doing so, it reminds us how much remains to be done if we are to ever fully understand the evolution of aging in natural populations.

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Dopamine: On the Threshold of Sleep

A new study examining the neural circuitry regulating sleep in *Drosophila* has identified a pair of dopamine neurons that signal to the fan-shaped body to suppress sleep. These neurons are separate from the dopamine neurons that regulate motivation, memory, and feeding, suggesting that independent populations of dopamine neurons regulate distinct behaviors.

Pavel Masek and Alex C. Keene

The neurotransmitter dopamine plays a central role in motivation, feeding, memory and sleep–wake regulation across phyla. Fruit flies mutant for the dopamine transporter, the primary target for cocaine and methamphetamine, have reduced sleep, revealing dopamine to be a potent suppressor of sleep [1]. Flies with reduced dopamine signaling have deficits in associative memory and feeding behaviors. How does a single transmitter regulate such diverse behavioral and cognitive processes? One possibility is through a diversity of receptors. Alternatively, the distinct effects of dopamine may be mediated through neural connectivity. The fly brain contains approximately 200 dopamine neurons with widespread projections throughout the central brain [2,3]. Identifying the specific dopamine neurons that modulate individual behavioral processes is difficult and limited by the availability of genetic tools capable of labeling individual classes of neurons.

In this issue of *Current Biology*, Liu *et al.* [4] identify two dopamine neurons that suppress sleep. The authors transgenically activate dopamine neurons in a temperature-dependent fashion by genetically expressing the heat-inducible cation channel transient-receptor-potential A1 (TRPA1) in small populations of dopamine neurons. Expressing TRPA1 under control of the Tyrosine Hydroxylase promoter allows for inducible activation of all dopamine neurons and results in a dramatic reduction in sleep [5]. Liu *et al.* generated driver lines using fragments of the Tyrosine Hydroxylase genomic locus to transgenically label and manipulate specific populations of dopamine neurons. One line labeled a single neuron in each of the

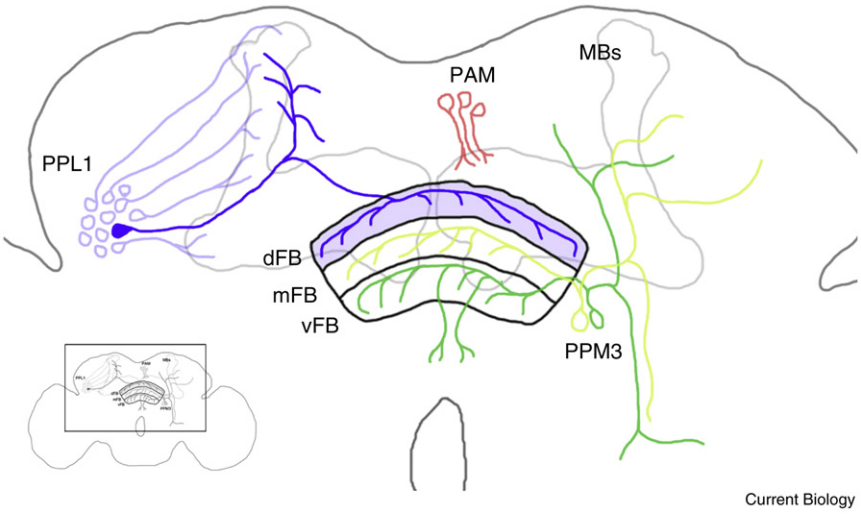

Figure 1. Schematic of sleep-suppressing dopamine neurons in the fly brain.

The fan-shaped body (FB) consists of three layers: dorsal (dFB), medial (mFB), and ventral (vFB). One dopamine neuron from each PPL1 cluster (PPL-dFB) suppresses sleep. Other PPL1 neurons innervate the mushroom bodies (MBs), which have previously been implicated in sleep and memory. Liu *et al*. [4] find that neurons from PPM3 neuronal cluster innervating vFB and mFB do not regulate sleep. Neurons from PAM cluster innervate medial lobes of MBs and signal positive and aversive reinforcement during learning. Inset depicts the magnified region within the fly brain.

protocerebral posterolateral (PPL1) clusters that was not labeled in other lines, and activation of these neurons resulted in reduced sleep. These findings suggest that a pair of neurons in the PPL1 cluster is capable of conferring sleep loss.

Careful morphological analysis revealed that the sleep-suppressing PPL1 neurons ramify throughout the *Drosophila* fan-shaped body (Figure 1), a region within the central complex that was previously shown to regulate sleep and locomotion [6,7]. The fruit fly genome encodes four dopamine receptors, and mutants for the type 1 dopamine receptor, *DopR*, have increased sleep [8]. *DopR* appears to be the primary dopamine receptor required for arousal, because loss of *DopR* fully suppresses the wake-promoting effects of dopamine [4]. *DopR* is expressed predominantly in the mushroom bodies and central complex, two brain regions previously implicated in sleep–wake regulation [9]. Liu *et al.* [4] found that selectively rescuing *DopR* in the fan-shaped body alone rescues the long-sleeping phenotype of these mutants. These results suggest that a pair of PPL1 dopamine neurons stimulate DopR, thereby elevating cAMP levels in the fan-shaped body. Flies with genetic manipulations that mimic the effects of enhanced cAMP signaling in the fan-shaped body also suppress sleep, fortifying the notion that dopamine signaling to the fan-shaped body promotes wakefulness [4].

There appears to be extensive redundancy in the neuroanatomy controlling sleep–wake regulation in *Drosophila*. A previous report found that rescue of *DopR* in the circadian pacemaker neurons labeled by Pigment Dispersing Factor (PDF) neuropeptide rescues sleep [8], suggesting that dopamine may regulate arousal through multiple loci. Identifying how distinct populations of *DopR* neurons regulate sleep will be critical for understanding dopamine function.

How does the activity of dopamine neurons contribute to sleep–wake regulation? One possibility is that wake-promoting dopamine neurons are more active during the day. To test this hypothesis the authors employed physiological imaging of PPL1 neurons during the subjective day and night. A neuropeptide fusion protein consisting of rat Atrial Naturetic Factor (ANF) fused to a green fluorescent protein reporter was used as a marker for imaging chronic neuronal activity in dopamine neurons [10]. These experiments suggest the PPL1 neurons innervating the fan-shaped body were more active during day than night [4]. Previous imaging studies of dopamine neurons using a cAMP-sensitive reporter have identified a dopamine-mediated increase in cAMP levels in circadian neurons [5]. While the effects of dopamine application on cAMP levels in the fan-shaped body was not measured by Liu *et al.* [4], this technique could be applied to examine dopamine-related signaling in the fan-shaped body.

Taken together, Liu *et al.* reveal neural circuitry that is sufficient for suppression of sleep in the fruit fly. These results are particularly important because they suggest a single pair of neurons is capable of regulating sleep–wake behavior. These findings present a number of questions that may provide critical insight into the neural circuitry regulating sleep. Which neurons activate the PPL1 fan-shaped body neurons and how are these neurons modulated in accordance with circadian cues and sleep need? Furthermore, the target neurons of the fan-shaped body remain unclear. The fan-shaped body is composed of three distinct layers of neurons that are differentially involved in visual memory and locomotion, suggesting these neurons may directly control locomotor and search behaviors. Future experiments addressing these questions may help to complete the dopamine-mediated, sleep-suppressing circuit in the fruit fly brain.

It is particularly interesting that single dopamine neurons appear to regulate distinct behaviors. A number of previous studies have elegantly demonstrated unique populations of dopamine neurons required for distinct memory tasks. Appetitive conditioning involving the pairing of sugar with a novel odor requires starvation prior to training [11]. PPL1 neurons expressing the receptor for the orexigenic transmitter, neuropeptide F, regulate appetitive conditioning by inhibiting appetitive memories in satiated flies [11]. These neurons are distinct from the sleep-regulating neurons because they exclusively innervate the mushroom bodies and do not affect sleep [4,11]. The pairing of electric-shock punishment with a novel odor is dependent on another population of PPL1 neurons that innervate the vertical lobes of the mushroom bodies [3]. The PAM population of dopamine neurons is critical for conveying aversive

reinforcement in olfactory conditioning [12] but a small subset of the same neurons also signal sugar reward to the mushroom bodies [13]. Taken together, these studies suggest at least three different populations of dopamine neurons innervate the mushroom bodies to confer distinct signals during classical conditioning and each of these dopamine neuron populations is distinct from the wake-promoting PPL1 neurons that innervate the fan-shaped body.

A recent study has also reported a single dopamine neuron that is necessary and sufficient for the induction of feeding behavior. Flies extend their proboscis (mouth) in response to sucrose presentation and this response is reduced in flies with genetically inactivated dopamine neurons. Activation of a single dopamine neuron in the subesophageal ganglion is sufficient to trigger proboscis extension in the absence of food presentation [14]. Therefore, small numbers, or even single dopamine neurons are capable of mediating distinguishable effects on behavior.

The identification of a sleep-suppressing role for PPL1 neuron signaling to the fan-shaped body reveals important neural circuitry underlying sleep–wake regulation. Future work examining how the activity of individual dopamine neurons that control distinct behavioral processes are regulated may reveal fundamental information about how the brain regulates and chooses among behaviors.

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Histones: Sequestered by Jabba in Fatty Storehouse

A paper in this issue shows that histones H2a and H2b are stored in lipid droplets in *Drosophila* embryos complexed with the protein Jabba. In *Jabba* mutant embryos, histones H2a and H2b are degraded but embryos survive by translating stored histone mRNA.

William F. Marzluff and Deirdre C. Tatomer

A critical feature of early embryogenesis in all metazoans is the need to provide the histone proteins in the oocyte that are required to assemble new chromatin during early embryogenesis. Immediately after fertilization the sperm chromatin is remodeled and maternal histone proteins replace the specialized sperm chromatin proteins. Subsequently both the maternal and paternal chromosomes replicate prior to the first cell division, followed by a series of zygotic divisions. In most embryos there is no transcription immediately after fertilization and in many species there are a substantial number of very rapid divisions that occur in the absence of zygotic transcription. These divisions result in a logarithmic increase in the amount of DNA and hence an exponential increase in the demand for histone protein for the assembly of newly synthesized chromatin. A number of distinct mechanisms have evolved to solve the problem of providing histones for early embryogenesis in different metazoans. In two well-studied systems, large amounts of histone proteins are provided by maternal stores of histone proteins and histone mRNAs: in *Xenopus* there are over 14 cell divisions resulting in a 10,000 cell embryo within 8 hours prior to initiation of zygotic transcription; and in *Drosophila* zygotic transcription of histone genes initiates in cycle 11 (about 1.5 hours after fertilization), when the syncytial embryo contains over 1,000 nuclei. In this issue of *Current Biology*, Welte and coworkers [1] show that, in *Drosophila* (and likely many insects), histones H2a and H2b are stored in lipid droplets and that proper storage is essential for the utilization of the stored histone for chromatin assembly.