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Sleep and wakefulness in *Drosophila melanogaster*

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Summary

Sleep is present and tightly regulated in every vertebrate species in which it has been carefully investigated, but what sleep is for remains a mystery. Sleep is also present in invertebrates, and an extensive analysis in *Drosophila melanogaster* has shown that sleep in fruit flies show most of the fundamental features that characterize sleep in mammals. In *Drosophila*, fly sleep consists of sustained periods of quiescence associated with an increased arousal threshold. Fly sleep is modulated by several of the same stimulants and hypnotics that affect mammalian sleep. Moreover, like in mammals, fly sleep shows remarkable interindividual variability. The expression of several genes involved in energy metabolism, synaptic plasticity, and the response to cellular stress varies in *Drosophila* between sleep and wakefulness, and the same occurs in rodents. Brain activity also changes in flies as a function of behavioral state. Furthermore, *Drosophila* sleep is tightly regulated in a circadian and homeostatic manner, and the homeostatic regulation is largely independent of the circadian regulation. After sleep deprivation recovery sleep in flies is longer in duration and more consolidated, as indicated by an increase in arousal threshold and fewer brief awakenings. Finally, sleep deprivation in flies impairs vigilance and performance. Because of the extensive similarities between flies and mammals, *Drosophila* is now being used as a promising model system for the genetic dissection of sleep. Over the last few years, mutagenesis screens have isolated several short sleeping mutants, a demonstration that that single genes can have a powerful effect on a complex trait like sleep.

Sleep is a universal phenomenon that occurs in every species studied so far, from mammals to insects (Tobler 2005). The function of sleep, however, remains unclear (Tobler 2005), but new genetic approaches can represent a promising strategy for identifying the mechanisms that control the need for sleep. Sleep is, at least to some extent, controlled by genetic factors. Several sleep disorders, from narcolepsy and somnambulism to REM sleep behavior disorder and fatal familial insomnia, are under genetic control (Dauvilliers et al. 2005). Moreover, the EEG correlates of sleep and waking are among the most heritable traits in humans (Linkowski 1999). Other aspects of normal sleep, including the duration of total sleep and of REM sleep, are also to some extent genetically determined (Dauvilliers et al. 2005). Forward genetic approaches represent a powerful strategy to assess the role of specific genes in regulating sleep duration or sleep need. In forward genetics, random mutations are tested for an effect on a specific phenotype. This approach requires no prior understanding of the biological process producing the phenotype and this has allowed researchers to investigate biological phenomena that seemed intractable due to their complexity. Since it is now clear that fruit flies sleep, *Drosophila melanogaster* can serve as a promising genetic tool for the investigation of sleep. Of course, the use of *Drosophila* as a model system to understand sleep relies on the ability to show that all the major features of sleep are shared between flies and mammals.

Fly sleep

Decades of research in circadian rhythms in *Drosophila* had clearly shown that fruit flies are active and move around during the day, much less so during the night. However, only in 2000 it became clear that the sustained periods of immobility during the night represented a sleep-like state and not just quiet wakefulness, because they were associated with a reversible increase in arousal threshold. Two independent groups of researchers provided the conclusive proof that *Drosophila* sleep indeed shares all the fundamental features of mammalian sleep (Hendricks et al. 2000; Shaw et al. 2000). Sleep is a complex integrative phenomenon that cannot be defined using one single criterion. Therefore in flies, like in mammals, sleep was defined using multiple criteria, the first of which is behavioral quiescence. Fly sleep behavior was first monitored using 3 methods: visual observation, an ultrasound activity monitoring system, and an automatic infrared system (Hendricks et al. 2000; Shaw et al. 2000). All provided similar results and confirmed that during the night flies show sustained periods of complete immobility that can last several hours. The most critical feature of sleep, however, is not immobility, but the presence of a reduced ability to respond to the external world. This decreased responsiveness is reversible, a feature that allows sleep to be distinguished from coma. Most importantly, an increase in arousal threshold distinguishes sleep from quiet wakefulness. Arousal thresholds in flies have been measured using vibratory, visual, or auditory stimuli (Shaw et al. 2000; Nitz et al. 2002; Huber et al. 2004). In all cases it was found that flies that had been moving around immediately before the stimulus readily responded to low and medium stimulus intensities. By contrast, flies that had been behaviorally quiescent for 5 min or more rarely showed a motor response, although they quickly responded when the stimulus intensity was increased. Thus, sleep can be operatively defined in flies as any period of behavioral quiescence longer than 5 minutes.

Sleep is highly regulated according to 2 processes: the circadian process and the homeostatic process (Borbely 1982). The circadian regulation is responsible for the change in sleep propensity that is tied to the time of day, with obvious adaptive advantages. Flies are diurnal animals and sleep mainly at night, even when kept in constant darkness (Shaw et al. 2000). In mammals the circadian and homeostatic regulation of sleep can be dissociated (Dijk and Lockley 2002) (Cajochen et al. 2002), at least to some extent. For instance, rats in which the central circadian clock has been destroyed by complete lesions of the suprachiasmatic nucleus no longer sleep in consolidated periods during the day (rats, unlike flies, are nocturnal) but rather show recurring episodes of sleep, lasting 1–3 hours each, across the 24-hour cycle (Mistlberger et al. 1983) (Tobler et al. 1983). When allowed to sleep after several hours of sleep deprivation, however, these rats still show a sleep rebound. A similar dissociation can be seen in flies in which the central circadian clock has been genetically destroyed by a mutation in one canonical circadian gene, e.g. *cycle*, *period*, or *Clock* (Shaw et al. 2000). These mutant flies sleep across the entire 24 hour period rather than just at night. However, after 24 hours of sleep deprivation, they still show a sleep rebound (Shaw et al. 2000).

The homeostatic process reflects sleep pressure depending on the length of prior waking: the longer one stays awake, the longer and more intensively one sleeps (Borbely 1982). This homeostatic component represents the essential aspect of sleep whose function remains mysterious. In flies, like in rodents and humans, sleep deprivation is followed by a sleep rebound characterized by an increase in the duration and/or in the intensity of sleep (Huber et al. 2004). Like in mammals, most of this sleep rebound occurs immediately after the end of the sleep deprivation period, is more pronounced after longer (12–24 hours) than after shorter (6 hours) periods of sleep loss, and the recovered sleep only represents a fraction of what was lost. Importantly, there is no increase in sleep duration when flies are subjected to 12 hours of the same stimulation during the day (when they are normally awake), ruling out non-specific effects. In mammals, sleep after sleep deprivation is also richer in slow-wave activity, a well-

characterized EEG marker of sleep intensity and sleep pressure, and is less fragmented, i.e. there are fewer periods of brief awakenings during sleep (Tobler 2005). In mammals, the increase in SWA after sleep deprivation is negatively correlated with the decrease in the number of brief awakenings (Franken et al. 1991). Sleep fragmentation as measured by the number of brief awakenings is also reduced in flies after sleep deprivation (Huber et al. 2004). Finally, in flies the recovery sleep that follows sleep deprivation is associated with a further increase in arousal threshold relative to baseline sleep, another indication that its intensity is increased (Huber et al. 2004). The ability of flies to move away from a noxious stimulus is impaired after 24 hours of sleep deprivation. This occurs despite the fact that sleep deprived flies, during testing, do not show an overall decrease in their spontaneous locomotor activity, ruling out non-specific effects of fatigue (Huber et al. 2004). It is still unknown whether sleep deprivation also affects the acquisition and/or the maintenance of memory, although it is clear that at least some short-sleeping mutants have impaired memory (see below).

Fly sleep seems to be sensitive to at least some of the same stimulants and hypnotics that modulate behavioral states in mammals. When given caffeine (Shaw et al. 2000) (Hendricks et al. 2000), modafinil (Hendricks et al. 2003), or amphetamines (Andretic et al. 2005), flies stay awake longer. By contrast, when fed with antihistamines, they go to sleep earlier (Shaw et al. 2000). Other similarities between fly and human sleep are present at the molecular level. Hundreds of transcripts change their expression in the rat, mouse, and sparrow brain between sleep and wakefulness, suggesting that in both birds and mammals sleep and wakefulness differ significantly at the molecular level (Cirelli et al. 2004) (Terao et al. 2006; Mackiewicz et al. 2007; Jones et al. in press). Using transcriptomics approaches such as mRNA differential display and microarray technology, which assess the expression of thousand of genes simultaneously, it was found that this is also the case in fruit flies (Cirelli et al. 2005a). As in rats, transcripts with higher expression in wakefulness and in sleep belong to different functional categories, and in several cases these groups overlap with those previously identified in rats. Wakefulness-related genes code for transcription factors and for proteins involved in synaptic plasticity, stress response, immune response, glutamatergic transmission, and carbohydrate metabolism. Sleep-related transcripts include glial genes and several genes involved in lipid metabolism.

In most mammalian studies, sleep is defined using behavioral as well as electroencephalographic (EEG) criteria: slow waves and spindles characterize non-rapid eye movement (NREM) sleep, while a high-frequency low amplitude EEG pattern with reduced muscle tone is present during REM sleep. Prolonged recordings of local field potentials (LFPs) from the medial part of the fly brain have been obtained in non-anaesthetized flies (Nitz et al. 2002). LFPs from awake, moving fruit flies are dominated by spike-like potentials (Nitz et al. 2002). These spikes largely disappear during the quiescent state when arousal thresholds are increased. Targeted genetic manipulations demonstrated that LFPs had their origin in brain activity and were not merely an artifact of movement or electromyographic activity (Nitz et al. 2002). Thus, like in mammals, wakefulness and sleep in fruit flies are accompanied by different patterns of brain electrical activity. However, the specific EEG features of mammalian sleep depend on the anatomy of the thalamocortical system, which does not exist in flies. It is not surprising, therefore, that sleep-related EEG events such as slow waves and spindles, which dominate the EEG during NREM sleep in birds and mammals, are not seen in flies. Also, electrical activity in neurons undergoes well characterized changes in mammals, including the occurrence, during NREM sleep, of slow (<1 Hz) oscillations in membrane potential. Whether such slow oscillations are also present in flies remains to be determined.

In the same fly, daily sleep amount and the timing of the major sleep phase are extremely consistent from one day to another (Cirelli 2003). The same parameters, however, vary significantly within individuals of the same fly population, even when age and housing

conditions are kept constant. The response to sleep deprivation also shows a strong interindividual variability, both in terms of sleep rebound as well as in terms of the effects on performance. This is why the characterization of sleep in any wild-type or mutant fly line requires the analysis of several individuals. Also, for the same reason, sleep cannot be measured at a population level, but needs to be quantified in individual flies. Recent studies in humans have also brought new attention to the issue of interindividual variability in sleep amount and in the response to sleep loss (Van Dongen et al. 2005). Importantly, in humans both sleep duration and the response to sleep deprivation show high intraindividual consistency, suggesting that they are trait-like (Tucker et al. 2007).

There are also features that distinguish fly sleep from mammalian sleep. Most animals including humans assume a typical posture when they go to sleep. Flies, however, do not appear to do so, at least not when their behavior is recorded inside the small glass tubes routinely used in sleep studies. Thus, based on the fly posture, it is not possible to distinguish quiet waking from sleep (unless one measures arousal thresholds). Several mammals clearly also change their posture when transitioning from NREM to REM sleep, due to the loss of muscular tone. As mentioned above, no study in flies so far has been able to detect different phases of sleep, similar to the NREM and REM phases in mammalian sleep, but a more accurate behavioral analysis, in more naturalistic conditions, has still to be performed.

Screening for sleep mutants in *Drosophila*

The demonstration that *Drosophila* sleeps is very important because it supports the notion that sleep fulfills some fundamental functions in many divergent animal species. However, *Drosophila* can also benefit sleep research by offering a powerful tool for the genetic dissection of sleep, just as it has benefited research on circadian rhythms. Over the last 7 years our laboratory has embarked on a large-scale mutagenesis screening in search for flies that need little sleep and/or do not show a sleep rebound after sleep deprivation. The final goal is to screen as many single-gene mutations as there are fly genes. So far, we have screened >15,000 mutant lines, many of them carrying a mutation in one single gene. The mutation was caused either by the insertion of a transposon in the fly genome (insertional mutagenesis), or by ethyl methanesulfonate (EMS, chemical mutagenesis). Insertional mutagenesis usually allows rapid identification of the mutated gene by sequencing the flanking sequences from one or both ends of the transposon insertion. However, transposons do not insert at random into the genome, but have preferred hot spots (Liao et al. 2000). Chemical mutagenesis with EMS, on the other hand, randomly induces small (point) mutations over the entire genome at a reasonable rate, but the molecular characterization of the gene of interest may be not as straightforward.

The daily amount of sleep in the mutant lines tested so far shows a normal distribution, with female flies for most lines sleeping between 400 and 800 min/day. Male flies, instead, sleep between 800 and 1100 min/day (Cirelli 2003). Twenty lines have so far qualified as “short-sleepers”, i.e. their daily sleep amount is less than 2 standard deviations from the mean of all mutant lines tested. Importantly, so far we have not identified any fly that does not sleep at all. Because of technical reasons, we currently measure sleep only in adult (at least 4-day old) flies. Any mutation that completely abolishes sleep may be lethal at an early stage of development, and thus would be missed in our current screening. Importantly, almost all mutant lines tested so far showed an increase in sleep duration and a decrease in sleep fragmentation after 24 hours of sleep deprivation. So far, we have identified only 4 lines, one of which is also a short sleeper line, which show no sleep rebound after 24 hours of sleep deprivation, suggesting that the absence of homeostatic response is a very rare phenotype.

In a recent study (Cirelli et al. 2005b) we found that flies carrying loss of function mutations in the gene *Shaker* sleep only 3–4 hours every day rather than 8–10 hours. The *Shaker* locus

encodes the alpha subunit of a tetrameric potassium channel that passes a voltage-activated fast-inactivating I_A current (Schwarz et al. 1988). Homologous channels in vertebrates have similar properties (Littleton and Ganetzky 2000) and, in both mammals and flies, I_A plays a major role in the control of membrane repolarization and transmitter release (Schwarz et al. 1988). *Shaker* mutations affect daily sleep amount but do not seem to affect the circadian and the homeostatic regulation of sleep. Specifically, short sleeping *Shaker* mutant flies still sleep mainly at night, and show a sleep rebound when sleep deprived (Cirelli et al. 2005b). *Shaker* mutant flies develop normally, and their escape response after sleep deprivation does not deteriorate as much as that of wild-type flies (Cirelli et al. 2005b). However, recent experiments in our laboratory show that learning and memory in these flies is impaired, although it is still unclear to what extent these deficits can be specifically ascribed to reduced sleep (Bushey et al. 2007). Also, short-sleeping *Shaker* mutants show an increased mortality, and this effect is at least partially dependent on reduced sleep (Cirelli et al. 2005b).

Hyperkinetic codes for a beta regulatory subunit that interacts with the alpha pore forming subunits coded by *Shaker*. In the presence of *Hyperkinetic* the amplitude of I_A increases, and its activation occurs more rapidly (Chouinard et al. 1995). Since *Shaker* activity is positively modulated by *Hyperkinetic*, and loss of function mutations of *Shaker* result in a decrease in daily sleep amount, we predicted that *Hyperkinetic* loss of function mutations would also result in reduced sleep. Indeed, we recently demonstrated that mutations in *Hyperkinetic* also cause a short sleeping phenotype (Bushey et al. 2007). Moreover, *Hyperkinetic* short sleeping flies also show impairment in learning and memory, and a reduced life span. As expected, the short sleeping phenotype in *Hyperkinetic* mutants is not as extreme as in *Shaker* mutants, because *Hyperkinetic* loss of function mutations reduce, but do not abolish, the I_A current. Why do *Shaker* mutations affect sleep duration so significantly? One possibility is that, by affecting an ion channel that controls membrane repolarization, they may be close to the core cellular mechanisms of sleep. In mammals, potassium channels are involved in the generation of sleep rhythms (Vyazovskiy et al. 2002) (Benington et al. 1995) (Espinosa et al. 2004). Importantly, *Shaker*-like channels are also present in mammals, and their role in the regulation of mammalian sleep need is currently under study. Kv3.1/Kv3.3-deficient mice show a decrease in daily sleep amount, although this is also associated with motor dysfunctions and hyperactivity (Espinosa et al. 2004). Based on sequence similarity, the closest mammalian homologues of the *Drosophila* *Shaker* are the alpha subunits of the Kv1 family, while the Kv2, Kv3, and Kv4 families are more distantly related ("Shaker-like"). Kv1 channels activate in the subthreshold voltage range in many cell types, and can act as extremely diverse regulators of neuronal excitability. In the supragranular layers of the rat cerebral cortex, for instance, most pyramidal cells contain different combinations of Kv1.1, Kv1.2, Kv1.3, and Kv1.4 subunits, localized in both the somatodendritic and the axonal compartments. In a recent study we found that young Kv1.2 (*Kcna2*)-deficient mice show a decrease in NREM sleep (Douglas et al. 2007). Unfortunately, all *Kcna2* null mice die within the first month of age after an episode of generalized seizure, and thus sleep could not be studied in adults. However, in other studies in rats we used intracortical injections of an antibody raised against the extracellular portion of the Kv1.2 channel, which was previously shown to block the Kv1.2 current by up to 70% (Zhou et al. 1998). This antibody abolished or reduced EEG signs of NREM sleep on the injected cortex for up to 12 hours after the injection. The effect was dose-dependent, reversible, and site-specific (Douglas et al. 2006). Whether human extreme short sleepers have mutations in voltage-dependent potassium channels remain to be determined. Intriguingly, however, in one case of Morvan's syndrome, a rare autoimmune disorder with central symptoms, marked sleeplessness has been associated with autoantibodies against voltage-dependent potassium channels that may have crossed the blood-brain barrier (Liguori et al. 2001).

Other *Drosophila* mutations able to reduce sleep affect genes coding for neuromodulators, or other proteins involved in the stress response and the PKA-CREB pathway. A mutation in the

Drosophila dopamine transporter (DAT) gene results in reduced sleep and increased activity levels (Kume et al. 2005). DAT is necessary for the uptake of dopamine uptake and modafinil, a waking promoting drug, targets the DAT orthologue in humans (Terao et al. 2005). Modafinil also has a waking-promoting effect on *Drosophila* but whether this effect is mediated by DAT in *Drosophila* has not been tested (Hendricks et al. 2003). Another mutation that reduces sleep duration in *Drosophila* affects the specific serotonin receptor *d5-HT1A*, while another serotonin receptor (*d5-HT1B*) is involved in maintaining circadian rhythm (Yuan et al. 2006). As mentioned before, as in mammals sleep deprivation in flies triggers a stress response that includes the upregulation of chaperones and heat shock proteins (Shaw et al. 2002; Naidoo et al. 2007). Mutations in stress response genes can either increase or decrease the response to sleep deprivation. For instance, loss of *Hsp83*⁰⁸⁴⁴⁵ results in an exaggerated increase in recovery sleep after sleep loss. By contrast, over-expression of *BiP* increases recovery sleep, while expressing a dominant negative form reduces recovery sleep (Naidoo et al. 2007). Presumably, these heat-shock proteins act at different steps in the stress response and thus their mutations can affect sleep at different levels. These results could suggest that sleep may be important to recover from some form of cellular stress associated with waking. However, so far mutations in stress response genes have not been reported to affect normal sleep, but only the rebound after sleep deprivation. Moreover, BiP in *Aplysia* is induced by learning (Kuhl et al. 1992), and in flies, rats and sparrows its expression increases also during physiological waking, not only after sleep deprivation, suggesting that its induction may not necessarily signal a pathological condition.

In both mice and flies there seems to be an inverse relationship between the activity of the cAMP-dependent protein kinaseA (PKA)-CREB pathway and daily sleep amount; increased PKA activation results in reduced daily sleep amounts (Hendricks et al. 2001; Graves et al. 2003). In recent screens using GAL4 drivers to induce expression of a constitutively active PKA transgene it was found that sleep amount may either increase or decrease depending on which neurons in the mushroom bodies PKA is expressed (Joiner et al. 2006). Specifically, with the 201Y GAL4 driver, in which PKA is mainly expressed in the γ (gamma) lobes and the core region of the α and β (alpha/beta) lobes, daily sleep amount was increased, while with line c309, in which PKA is expressed in the α (alpha), β (beta), and γ (gamma) lobes but not in the core region, sleep was decreased. Since PKA has many targets, it is unclear whether the effects on sleep are mediated directly through the phosphorylation of CREB or of other targets, such as the *Shaker* channel itself (Drain et al. 1994). When a constitutive PKA transgene is expressed throughout the mushroom bodies or pan-neuronally, sleep is reduced, and it remains unclear how the output from the mushroom bodies is regulating sleep duration. Expressing transgenic channels in the mushroom bodies to suppress or increase synaptic output results in increased and decreased sleep time, respectively, a finding that suggests that the activation of PKA decreases sleep by increasing synaptic output from the mushroom bodies (Joiner et al. 2006). However, the chemical ablation of the mushroom bodies decreases sleep time (Pitman et al. 2006). Clearly future experiments are needed to clarify the mechanisms by which PKA affects sleep duration via the mushroom bodies.

Present and future challenges

An efficient genetic screen relies on being able to screen as many mutant lines as possible. Unfortunately, sleep is a complex trait that is determined by many environmental and genetic factors and as a result has high interindividual variability. Thus, in a screen for fly sleep mutants, many flies that inherit a putative mutation have to be tested over many generations to confirm that the phenotype is inherited. Then, genetic mapping must prove that the phenotype maps to a single locus. Finally, multiple mutant alleles for a given locus must be identified to confirm that mutations within the gene produce a sleep phenotype, and to provide insight into how the mutations are altering sleep. Our effort to characterize the *Shaker* mutation *minisleep*

(*Shaker^{mns}*) highlighted potential problems with this process. At first, we found that although *Shaker^{mns}* was short sleeping, other *Shaker* loss-of-function alleles did not produce a sleep phenotype. This led to the erroneous conclusion that in *Shaker^{mns}* flies the *Shaker* mutation was a second site mutation that had nothing to do with the short sleeping phenotype. We realized this conclusion was wrong after using classical genetic techniques, which confirmed that the *minisleep* phenotype did indeed map to the *Shaker* locus. This prompted us to out-cross the other *Shaker* loss-of-function alleles, the ones that previously had shown no change in sleep duration, to remove genetic modifiers that we hypothesized could be suppressing the short sleeping phenotype. Indeed, after out-crossing, all the *Shaker* loss-of-function alleles produced a short sleeping phenotype. Thus, modifiers can accumulate in mutant stocks (especially in long-standing stocks that have been maintained in the laboratory for years) and can suppress the sleep phenotype. We think this occurs because the short sleeping phenotype, at least in some cases, is associated with reduced longevity and impaired memory (Bushey et al. 2007). This is why we now always test siblings, since siblings share a common genetic background and have an equal chance of inheriting a given modifier, provided that the modifier is not linked to the gene of interest. Furthermore, siblings develop in the same environment and are only separated for testing after developing into adults. Thus, siblings that inherit the mutation can be compared to those that do not, and any change in the sleep phenotype can be attributed to the mutation rather than to other variables. In contrast, when sleep is compared across different stocks, difference in genetic background and rearing conditions (e.g. crowding) may account for major differences in the sleep phenotype.

The potential confounding effects of the genetic background should always be considered. A fly line carrying the mutation of interest must inevitably be crossed to other stocks in order to study the mutation's effect on sleep. Stocks, including "wild-type" stocks such as *Canton-S* (*CS*) and *w^{CS10}* that originated from a common stock, diverge widely in total sleep amount, and this difference does not result from a single mutation but rather from slight differences in a number of alleles at many difference loci (the so-called "genetic background"). Although quantitative trait loci analysis can roughly map these loci, this usually does not lead to the identification of "major" genes. The identification is difficult because each allele usually produces only a slight phenotypic difference. Thus, if the sleep phenotype is compared using different stocks, without correcting for the genetic background, positive results may be found that are due to the combined effect of the other loci rather than to the mutation of interest. Genetic background can also have a strong effect on the penetrance of a mutant phenotype. For example, the *w¹¹¹⁸* stock shows little sleep rebound after 24 hours of sleep deprivation, while the *CS* stock shows a strong rebound. Therefore, the *CS* stock represents a much better choice when one screens for a rebound phenotype. Some mutagenesis screens use isogenic strains to reduce variability in sequence and phenotype between progeny. Isogenic strains are assumed to have reduced phenotypic variability because all the individuals have the same genomic sequence. However, these inbred isogenic lines do not necessarily produce more uniform progeny compared to hybrid lines (McLaren 1999). In fact, traits can be selected for and maintained in an isogenic background, suggesting that there is an epigenetic mechanism that results in variability on which selection can act (Sollars et al. 2003). Therefore, isogenic backgrounds do not necessarily prevent modifiers from occurring and suppressing a sleep phenotype, since modifiers can also arise from inheritable epigenetic changes within the stock. This is why we think that comparing siblings reared in a common environment and that have an equal chance of inheriting allelic variation produced by sequence or epigenetic difference is the more reliable option.

Lastly, sleep is considered to be more important for the central nervous system than for other tissues. Genetic screens that could specifically study the effects of mutations on the fly brain rather than throughout the fly body would be a great advantage, because they would avoid pleiotropic effects produced by a given mutation. Gene products are often co-opted in many

diverse functions throughout the body and therefore their loss produces multiple phenotypes. Genetic techniques such as mosaic analysis with a repressible cell marker (MARCM) demonstrates that mutant clones can be generated in neural cells (Lee and Luo 2001). However, so far MARCM has been able to generate only random clones, and the inability to generate consistent clones has remained an obstacle in developing this type of tissue-targeted screen.

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