

# foraging alters resilience/vulnerability to sleep disruption and starvation in *Drosophila*

Jeffrey Donlea<sup>a,1</sup>, Averi Leahy<sup>a,1</sup>, Matthew S. Thimman<sup>a</sup>, Yasuko Suzuki<sup>a</sup>, Bryon N. Hughson<sup>b</sup>, Marla B. Sokolowski<sup>b</sup>, and Paul J. Shaw<sup>a,2</sup>

<sup>a</sup>Department of Anatomy & Neurobiology, Washington University in St. Louis, St. Louis, MO 63104; and <sup>b</sup>Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada M5S 3B2

Edited\* by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved January 4, 2012 (received for review August 7, 2011)

Recent human studies suggest that genetic polymorphisms allow an individual to maintain optimal cognitive functioning during sleep deprivation. If such polymorphisms were not associated with additional costs, selective pressures would allow these alleles to spread through the population such that an evolutionary alternative to sleep would emerge. To determine whether there are indeed costs associated with resiliency to sleep loss, we challenged natural allelic variants of the *foraging* gene (*for*) with either sleep deprivation or starvation. Flies with high levels of Protein Kinase G (PKG) (*for<sup>R</sup>*) do not display deficits in short-term memory following 12 h of sleep deprivation. However, short-term memory is significantly disrupted when *for<sup>R</sup>* flies are starved overnight. In contrast, flies with low levels of PKG (*for<sup>S</sup>*, *for<sup>S2</sup>*) show substantial deficits in short-term memory following sleep deprivation but retain their ability to learn after 12 h of starvation. We found that *for<sup>R</sup>* phenotypes could be largely recapitulated in *for<sup>S</sup>* flies by selectively increasing the level of PKG in the  $\alpha/\beta$  lobes of the mushroom bodies, a structure known to regulate both sleep and memory. Together, these data indicate that whereas the expression of *for* may appear to provide resilience in one environmental context, it may confer an unexpected vulnerability in other situations. Understanding how these tradeoffs confer resilience or vulnerability to specific environmental challenges may provide additional clues as to why an evolutionary alternative to sleep has not emerged.

Although sleep is a behavioral state that is conserved across a diverse range of species, the biological functions of sleep remain unknown. Sleep deprivation (SD) has been shown to negatively impact cognition, but individual responses to sleep loss can vary significantly within a population (1). Recent studies suggest that a portion of this variability may be influenced by genetic factors (2). For example, polymorphisms for PERIOD 3 (*PER3*), a circadian clock gene, can predict the magnitude of cognitive impairment and sleep homeostasis in response to a night of SD in humans (2). Although these genetic contributions may attenuate impairments following SD, the tradeoffs associated with resistance to sleep loss remain unknown. Presumably, the potential costs must be substantial. Thus, it is likely that the price of protection from sleep loss that can be conferred by allelic variation in one environment may induce a cost when manifested in a different environment. To date, putative costs of resiliency to sleep loss have not been identified in humans or any model organism.

*foraging* (*for*), which codes for Protein Kinase G (PKG), is maintained in wild-type populations as a genetic polymorphism that results in either higher or lower levels of PKG activity (3). The allele associated with higher levels of PKG (“rover”; *for<sup>R</sup>*) results in larvae with longer foraging trails between food patches, whereas the allele associated with lower levels of PKG (“sitter”; *for<sup>S</sup>*) results in larvae with shorter foraging trails; a mutant of *for* (*for<sup>S2</sup>*) generated in the *for<sup>R</sup>* background also displays shorter foraging trails. Different foraging patterns appear beneficial in discrete situations, so neither allele has achieved a consistent advantage, suggesting an explanation for their persistence over time (4). Interestingly, *for* is highly pleiotropic and is known to influence many behaviors in multiple species (5), including sleep (6, 7) and learning and memory (8), to name only a few. With

respect to learning and memory, recent studies have shown that *for<sup>R</sup>* flies perform better on short-term memory tasks than *for<sup>S</sup>* flies, whereas *for<sup>S</sup>* flies have better long-term memory acquisition (4). These differences suggest that the *for* alleles may confer strikingly different strategies for survival, with clear advantages and disadvantages in distinct environments (8, 9).

SD is known to result in robust cognitive impairments in humans (10), rodents (11), bees (12), and flies (13, 14). However, the extent to which prolonged waking will result in cognitive impairments is strongly influenced by the environmental context. For example, although starvation is known to induce wakefulness in many animals (15, 16), including flies (17, 18), recent studies from our laboratory indicate that wakefulness induced by starvation is not accompanied by cognitive impairments (18). Given that the *foraging* gene has been implicated in memory and sleep as well as energy storage and responses to food deprivation, it is likely that flies with the naturally existing *foraging* polymorphisms will differ in their ability to maintain cognitive functioning during sleep loss. Indeed, a recent study has reported that *foraging* alters the amount of waking observed during starvation (17). However, neither sleep homeostasis, survival, nor cognitive behaviors were evaluated in *for<sup>R</sup>* and *for<sup>S</sup>* flies following starvation. As a consequence, it remains unclear whether the alternate waking strategies exhibited by *for<sup>R</sup>* and *for<sup>S</sup>* flies result in functional outcomes that may provide a selective advantage or disadvantage during food loss. Because the physiological demands of SD are likely to differ from those observed during starvation, it is unlikely that the molecular mechanisms that allow the animal to succeed in one environment will be effective in the other. Thus, we hypothesized that behavioral responses of *foraging* allelic variants that may confer an advantage to SD would be deleterious during starvation.

## Results

We hypothesized that polymorphisms in *foraging* would influence the response to SD as measured by both sleep homeostasis, the increase in sleep seen following sleep loss, and short-term memory. Because diet strongly modulates the behavior of the *foraging* alleles, we first asked whether *for<sup>R</sup>* flies would sleep significantly longer than *for<sup>S2</sup>* mutants when tested under our laboratory conditions as described previously (7). As seen in Fig. S1, under our dietary conditions, *for<sup>R</sup>* flies sleep significantly longer than *for<sup>S2</sup>* mutants.

Next, we exposed *for<sup>R</sup>*, *for<sup>S</sup>*, and *for<sup>S2</sup>* flies to 12 h of SD during their primary sleep period using the sleep-nullifying apparatus. As seen in Fig. 1A, *for<sup>R</sup>* flies did not compensate for lost sleep during 48 h of recovery, whereas both *for<sup>S</sup>* flies and *for<sup>S2</sup>* mutants

Author contributions: J.D., A.L., M.S.T., M.B.S., and P.J.S. designed research; J.D., A.L., M.S.T., Y.S., B.N.H., and P.J.S. performed research; B.N.H. contributed new reagents/analytic tools; J.D., A.L., Y.S., and P.J.S. analyzed data; and J.D., A.L., M.B.S., and P.J.S. wrote the paper.

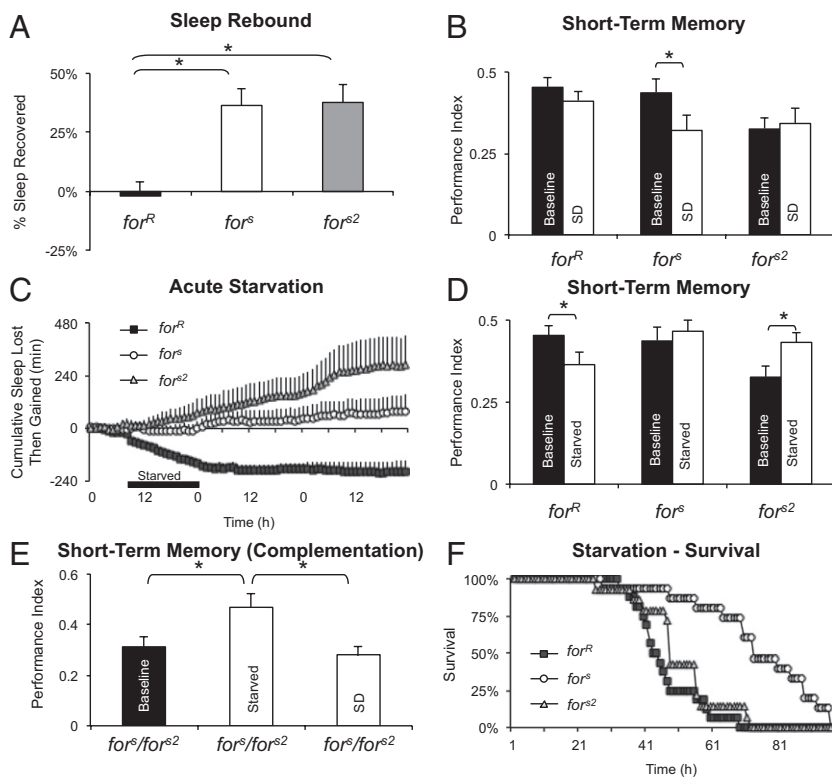
The authors declare no conflict of interest.

\*This Direct Submission article had a prearranged editor.

<sup>1</sup>J.D. and A.L. contributed equally to this work.

<sup>2</sup>To whom correspondence should be addressed. E-mail: shawp@pcg.wustl.edu.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1112623109/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1112623109/-DCSupplemental).



**Fig. 1.** *foraging* differentially confers resilience/vulnerability to SD and starvation. (A) *for<sup>R</sup>* flies do not compensate for 12 h of SD with a subsequent increase in sleep, whereas both *for<sup>S</sup>* and *for<sup>S2</sup>* mutants exhibit a sleep rebound typical of *Cs* flies. % sleep recovered is calculated for each individual as a ratio of the minutes of sleep gained above baseline during 48 h of recovery divided by the minutes of sleep lost during SD; \* $P < 0.05$ , modified Bonferroni test. Data are presented as mean  $\pm$  SEM. (B) STM is impaired in *for<sup>S</sup>* flies following 12 h of SD, whereas *for<sup>S2</sup>* mutants display impairments both during baseline and following SD. In contrast, *for<sup>R</sup>* flies maintain normal STM following SD. (C) When placed in starvation before lights out, *for<sup>R</sup>* flies display significantly less sleep than during the previous baseline night and do not exhibit a sleep rebound when placed back onto food the following morning. Neither *for<sup>S</sup>* flies nor *for<sup>S2</sup>* mutants respond to starvation with an increase in waking. Cumulative sleep lost or gained during starvation. (D) STM is impaired in *for<sup>R</sup>* flies when waking is induced by starvation. *for<sup>S</sup>* flies maintain STM following a night of starvation. Surprisingly, *for<sup>S2</sup>* mutants have normal STM following a night of starvation. (E) STM is impaired in *for<sup>S</sup>/for<sup>S2</sup>* flies under baseline conditions compared with starved siblings. Sleep-deprived *for<sup>S</sup>/for<sup>S2</sup>* flies show no further STM impairments relative to baseline. (F) *for<sup>S</sup>* flies survive longer than *for<sup>R</sup>* and *for<sup>S2</sup>* flies during chronic starvation ( $n > 27$  per group).

displayed a sleep rebound similar to that previously seen in *Canton-s* (*Cs*) flies (19–21). The lack of a homeostatic response seen in *for<sup>R</sup>* flies may represent either an adaptation that allows animals to better withstand the negative effects of waking, or it may indicate that *foraging* disrupts regulatory processes, thereby preventing flies from obtaining needed sleep. Because deficits in short-term memory are a robust consequence of sleep loss (13, 22, 23), we evaluated short-term memory (STM) using aversive phototaxic suppression (APS) in *for<sup>R</sup>*, *for<sup>S</sup>*, and *for<sup>S2</sup>* flies following 12 h of SD. In the APS, flies are individually placed in a T maze and allowed to choose between a lighted and darkened chamber (13, 24). During 16 trials, flies learn to avoid the lighted chamber, which is paired with an aversive stimulus (quinine/humidity). The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials of the 16-trial test (13, 25). As seen in Fig. 1B, *for<sup>R</sup>* flies maintain their ability to learn following SD, whereas *for<sup>S</sup>* flies are significantly impaired; *for<sup>S2</sup>* mutants showed impaired performance in the APS both under baseline conditions and following SD. *for<sup>R</sup>*, *for<sup>S</sup>*, and *for<sup>S2</sup>* flies did not differ in sensory thresholds as measured by either the photosensitivity index (PI; percentage of photopositive choices in 10 trials in the absence of quinine) or the quinine sensitivity index (QSI; time in seconds flies reside on the non-quinine side of a chamber) (Table S1) (13, 25). Given that *for<sup>R</sup>* flies maintain their ability to learn following 12 h of SD and do not appear to be sleepy, as indicated by the absence of a sleep rebound, *for<sup>R</sup>* flies are considered to be resistant to SD. In contrast, both *for<sup>S</sup>* and *for<sup>S2</sup>* flies remain vulnerable to the negative effects of extended waking as measured by learning deficits and an increased sleep rebound.

Interestingly, the deleterious effects of extended waking are absent when waking is induced by starvation (18). Given that *foraging* alters the response to food deprivation, we hypothesized that *for<sup>R</sup>*, *for<sup>S</sup>*, and *for<sup>S2</sup>* mutants would show different vulnerabilities to waking induced by starvation. As seen in Fig. 1C, when *for<sup>R</sup>* flies are placed in recording tubes with agar and water (starvation), they exhibit an immediate and sustained increase in waking behavior and show no evidence of a sleep rebound when placed back on

their standard diet 12 h later. Interestingly, whereas the wake-promoting effects of starvation are absent in *for<sup>R</sup>* flies as previously described (17, 18), *for<sup>S2</sup>* mutants respond to starvation with a significant increase in sleep (Fig. 1C). If *for<sup>R</sup>* flies are resistant to the negative effects of waking induced by starvation, they should maintain their ability to learn in the APS as they did following SD. However, in contrast to waking induced by SD, *for<sup>R</sup>* flies are impaired following waking induced by starvation (Fig. 1D). Surprisingly, *for<sup>S2</sup>* mutants, which exhibit impaired short-term memory both under baseline conditions and after SD, recover their ability to learn when starved; previous studies have shown that neither SD nor starvation alters PI or QSI (13, 18, 25, 26). Thus, *for<sup>S2</sup>* mutants sleep more and display normal cognitive behavior following starvation, whereas *for<sup>R</sup>* flies display an unexpected vulnerability in short-term memory when waking is induced by the absence of food. Consistent with previous results (17, 18), SD and starvation likely invoke distinct physiological responses even though each manipulation produces an increase in waking.

Resilience to sleep loss is indicated by the ability to maintain optimal performance after sleep disruption. Thus, although baseline learning in *for<sup>S2</sup>* mutants is at the level observed in memory mutants (13, 25), neither *for<sup>S2</sup>* mutants nor *for<sup>R</sup>* flies, which are in the same genetic background, show performance decrements following sleep loss. With that in mind, we conducted a complementation test to examine learning in *for<sup>S</sup>/for<sup>S2</sup>* flies under baseline and in response to both SD and starvation. As seen in Fig. 1E, *for<sup>S</sup>/for<sup>S2</sup>* flies do not learn under baseline or after SD, but display STM after starvation. These results, along with the gain- and loss-of-function data presented below, indicate that it is the levels of *foraging*, rather than genetic background, which most likely account for differences in learning following SD and starvation.

Although waking up to forage during starvation would enhance the opportunity to find food, it requires additional energy expenditure. In contrast, sleeping would minimize the ability to find food but would likely conserve energy. Thus, we asked whether the alternate behavioral strategies exhibited by *for<sup>R</sup>* and *for<sup>S2</sup>* mutants would be associated with changes in survival during starvation. The average difference in the LD<sub>50</sub> in hours to

starvation between *for<sup>R</sup>* and *for<sup>s2</sup>* mutants was  $10.25 \pm 3.19$  ( $t = 2.306$ ,  $P = 0.01$ , one-sample  $t$  test,  $n = 4$  replicates); a representative example of survival during starvation is shown in Fig. 1E. Thus, whereas *for<sup>R</sup>* flies appear resistant to the behavioral consequences of SD, *for<sup>s2</sup>* flies appear more suited to withstand the challenge of overnight starvation.

Given that sleep plays a role in memory consolidation (27) and that the *foraging* polymorphism has been shown to independently alter both sleep and memory (7, 8), we examined the relationship between sleep and plasticity in *for<sup>R</sup>*, *for<sup>s</sup>*, and *for<sup>s2</sup>* flies. Previous studies have shown that enriched social environments induce synaptic elaboration in mammals and flies and that these changes are followed by several days of increased sleep (28–30). Thus, we evaluated sleep in *for<sup>R</sup>*, *for<sup>s</sup>*, and *for<sup>s2</sup>* flies after they had been exposed to either social enrichment, which consists of ~60 flies maintained in a 50-mL vial, or social isolation, which consists of flies being housed individually in TriKinetics tubes, for 5 d (31, 32). Surprisingly, neither *for<sup>s</sup>* nor *for<sup>s2</sup>* flies, which have long-term memory (LTM) using olfactory conditioning (8), display an increase in sleep following social enrichment (Fig. 2A). In contrast, *for<sup>R</sup>* flies, which have impaired LTM using olfactory conditioning, maintain their ability to increase sleep following social enrichment (Fig. 2A). Together, these data extend results with STM and LTM by showing that *foraging* plays a role in an additional type of plasticity, and further suggest that *foraging* may play a unique role when plasticity is induced in a social context.

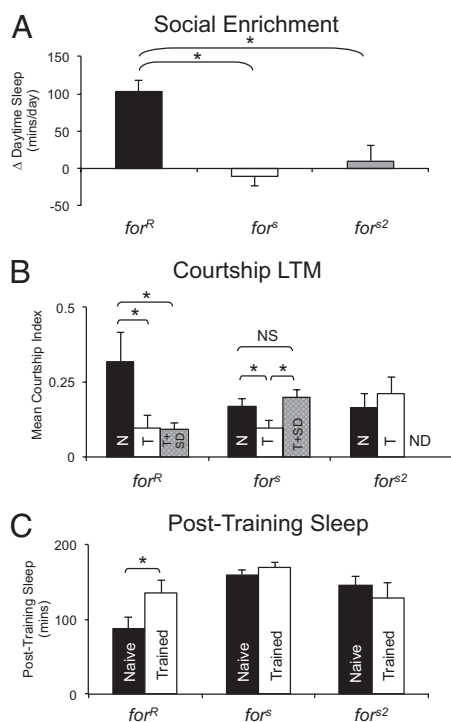
To further test this hypothesis, we evaluated LTM in male flies using a spaced training protocol in a courtship conditioning assay that results in decreased courtship behavior for at least 48 h after

training (31, 32). As seen in Fig. 2B, *for<sup>R</sup>* and *for<sup>s</sup>* flies display a significant reduction in courtship 48 h following spaced training (T) compared with their naïve siblings (N), indicating that they developed LTM. In contrast, *for<sup>s2</sup>* males show no reduction in courtship, indicating that they have impaired memory consolidation (Fig. 2B, Right). Note that whereas naïve courtship was low in *for<sup>s</sup>* males, it was not so low as to preclude the development of LTM. Moreover, naïve courtship was also low in *for<sup>s2</sup>* mutants, which are in the same genetic background as *for<sup>R</sup>*. Thus, it is likely that the reduced level of naïve courtship is due to the *foraging* gene and not due to genetic background. The observation that *for<sup>R</sup>* flies show both an increase in sleep in response to social enrichment and LTM following courtship conditioning suggests that *foraging* may be particularly relevant for plasticity induced in a social context.

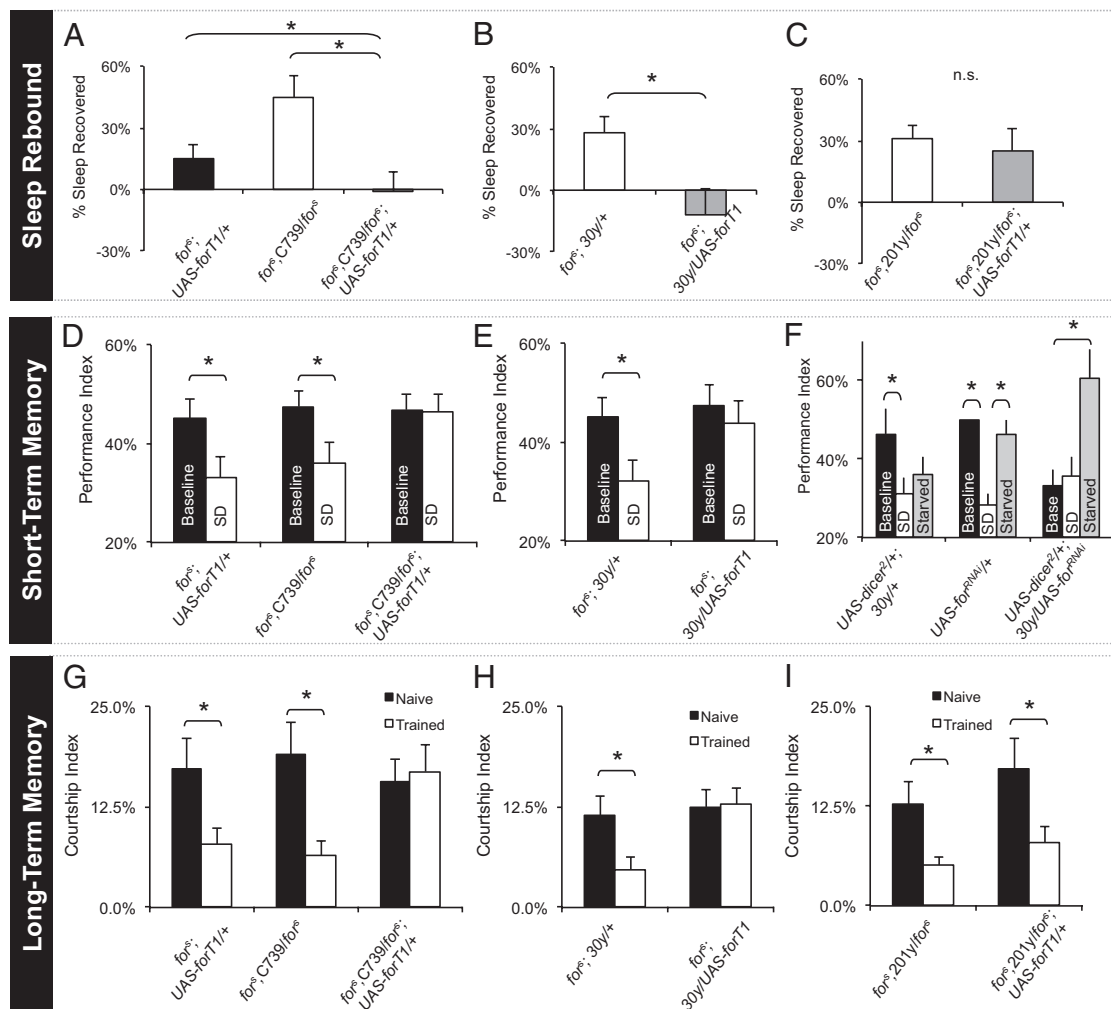
We have previously shown that sleep is increased following courtship conditioning in *Cs* flies and that LTM is disrupted if flies are sleep-deprived immediately following training (31). As seen in Fig. 2C, male *for<sup>R</sup>* flies sleep significantly more following spaced training than their naïve siblings. Similarly, *for<sup>s2</sup>* mutants, which did not develop LTM, did not increase their sleep following training, consistent with previous reports that courtship behavior in the absence of LTM formation does not alter sleep (31). Given that *for<sup>s</sup>* flies developed an LTM, it is unclear why they did not show an increase in posttraining sleep (Fig. 2C). However, one explanation may be that the changes in the behavior of *for<sup>s</sup>* flies shown in Fig. 2B were too small to effectively induce changes in sleep. We next asked whether posttraining SD would disrupt memory consolidation in *for<sup>R</sup>* and *for<sup>s</sup>* flies. Interestingly, 4 h of SD immediately following spaced training (T+SD) did not disrupt LTM in *for<sup>R</sup>* flies, whereas LTM was disrupted in *for<sup>s</sup>* flies. Thus, *foraging* appears to allow memory consolidation to proceed in the absence of sleep.

Given that the mushroom bodies (MBs) modulate both sleep and memory (13, 33, 34), we hypothesized that *for* signaling in the MBs would phenocopy *for<sup>R</sup>* and confer resistance to SD. Sleep homeostasis and performance in the APS were evaluated following 12 h of SD in flies overexpressing *for* in the MBs of otherwise *for<sup>s</sup>* homozygous background. As seen in Fig. 3A and B, when *for* is overexpressed primarily in the  $\alpha/\beta$  lobes of the MB using the *c739* or *30y* GAL4 drivers, sleep rebound is significantly attenuated. Thus, expressing *for* in the MBs recapitulates the sleep rebound phenotype observed in *for<sup>R</sup>* flies. Interestingly, overexpression of *for* using the *201y* GAL4 driver, which expresses predominantly in the  $\lambda$  lobes and only weakly in the  $\alpha/\beta$  lobes, does not significantly alter sleep rebound (Fig. 3C). As mentioned above, a low sleep rebound could represent either an adaptation that allows animals to better withstand the negative effects of waking, or a disruption in regulatory processes that prevent flies from obtaining needed sleep. Consistent with the *for<sup>R</sup>* phenotype described above, overexpressing *for* using *c739* or *30y* in an otherwise *for<sup>s</sup>* background also prevented deficits in short-term memory following SD (Fig. 3D and E). *201y/+; for<sup>s</sup>* control flies were altered in the APS under baseline conditions, such that the effect of MB  $\gamma$ -lobe overexpression using *201y* on short-term memory after SD could not be assessed. No differences in sensory thresholds were observed between genotypes. To determine whether reducing *foraging* within the MBs would phenocopy *for<sup>s2</sup>* mutants, we expressed *UAS-for<sup>RNAi</sup>* using *UAS-dicer<sup>2</sup>;30y-GAL4*. As seen in Fig. 3F, *UAS-dicer<sup>2</sup>;30y-GAL4/+>UAS-for<sup>RNAi</sup>* flies, but not parental controls, showed disrupted STM during baseline and after SD, whereas STM was restored following starvation. These results are similar to those observed in *for<sup>s2</sup>* mutants. These data indicate that *for* activity in the mushroom bodies, particularly the MB  $\alpha/\beta$  lobes, recapitulates the sleep phenotypes observed in *for<sup>R</sup>* and *for<sup>s2</sup>* flies.

A previous report indicates that *for<sup>R</sup>* flies and flies overexpressing *for* within the MBs using *c739*, *30y*, and *201y* GAL4 drivers have impaired LTM following olfactory conditioning (8). However, the data presented above indicate that *for<sup>R</sup>* flies can generate LTM following courtship conditioning. To further define



**Fig. 2.** Sleep deprivation does not block LTM in *for<sup>R</sup>* flies. (A) *for<sup>R</sup>* females increase sleep following social enrichment relative to isolated siblings, whereas *for<sup>s</sup>* and *for<sup>s2</sup>* females do not.  $*P < 0.05$ , modified Bonferroni test. Data are presented as mean  $\pm$  SEM. (B) *for<sup>R</sup>* flies have intact LTM that is not disrupted when sleep-deprived for 4 h immediately after training (T+SD). Trained *for<sup>s</sup>* flies have intact LTM during baseline but are impaired when sleep-deprived following training. *for<sup>s2</sup>* mutants have impaired LTM; courtship was not evaluated in *for<sup>s2</sup>* flies following SD (ND). NS, nonsignificant.  $*P < 0.05$ , modified Bonferroni test. (C) Following spaced training for courtship conditioning, *for<sup>R</sup>* males, but not *for<sup>s</sup>* or *for<sup>s2</sup>* flies, sleep significantly more than their naïve siblings;  $*P = 0.03$ .



**Fig. 3.** Expressing *foraging* in the MB confers resilience to sleep deprivation. (A–C) When *UAS-*for** is expressed using *c739-GAL4/for<sup>R</sup>* (A) and *30y-GAL4/for<sup>R</sup>* (B) drivers, no sleep rebound is observed after 12 h of SD. Parental lines display a sleep rebound. Interestingly, *w; for<sup>R</sup>; 201y/for<sup>R</sup>; UAS-*for** flies (C) and their parental controls exhibit a sleep rebound; \* $P < 0.05$ , modified Bonferroni test. Data are presented as mean  $\pm$  SEM. n.s., nonsignificant. (D and E) As expected, *for<sup>R</sup>; c739/for<sup>R</sup>*, *w; for<sup>R</sup>; UAS-*for*+*, and *w; for<sup>R</sup>; 30y* parental lines display significant reductions in STM following 12 h of SD, whereas both *w; for<sup>R</sup>; c739/for<sup>R</sup>; UAS-*for*+* (D) and *w; for<sup>R</sup>; 30y/UAS-*for** (E) flies retain STM following SD. (F) During baseline, *UAS-*dicer*<sup>2/+</sup>; 30y/UAS-*for*<sup>RNAi</sup>* flies show deficits in STM, whereas both parental controls learn; performance remains low after SD in all genotypes. However, *UAS-*dicer*<sup>2/+</sup>; 30y/UAS-*for*<sup>RNAi</sup>* flies display STM following starvation. (G–I) Courtship conditioning fails to induce LTM in *w; for<sup>R</sup>; c739/for<sup>R</sup>; UAS-*for*+* (G) and *w; for<sup>R</sup>; 30y/UAS-*for** (H) but is intact in the parental lines. *w; for<sup>R</sup>; 201y/for<sup>R</sup>; UAS-*for*+* flies and parental controls have intact LTM (I).

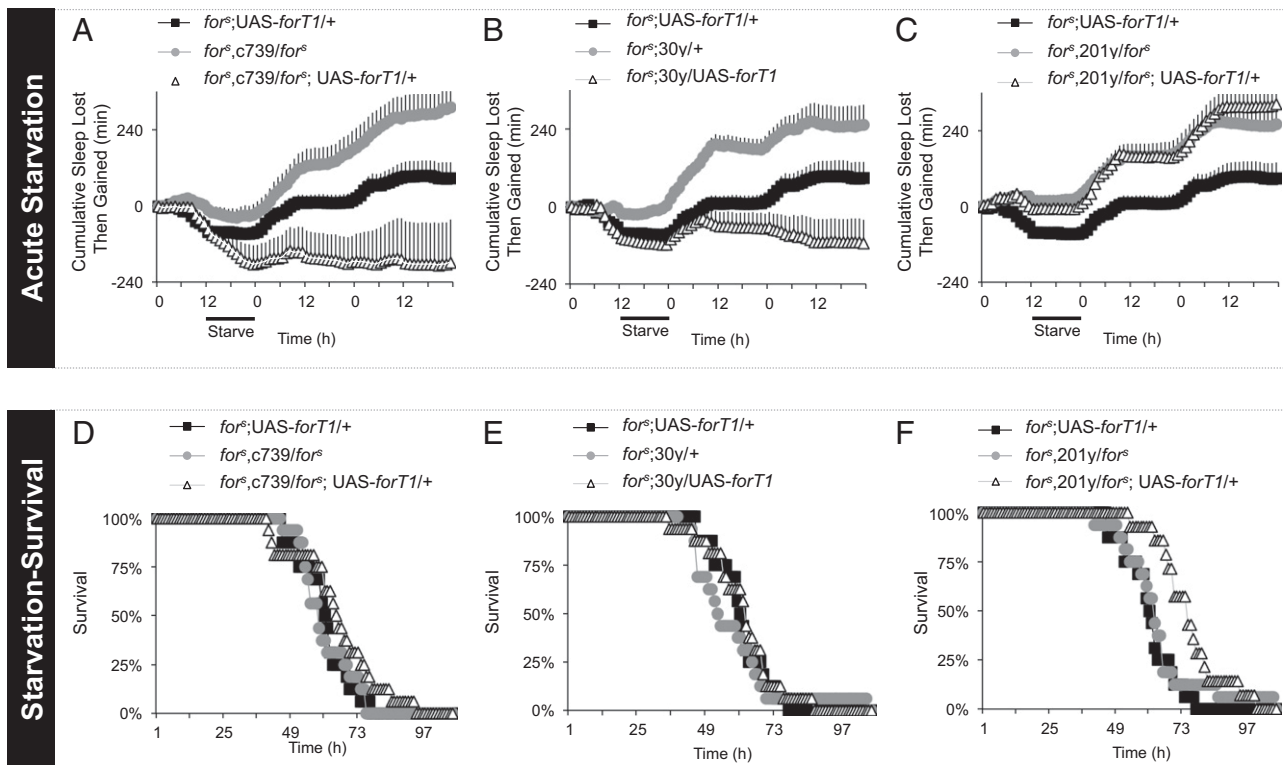
the role of *foraging* in LTM induced by courtship conditioning, we expressed *for* in the MBs of an otherwise *for<sup>R</sup>* background. Consistent with the results reported for olfactory conditioning, expressing *for* in the MB  $\alpha/\beta$  lobes using *c739* or *30y* significantly disrupted LTM (Fig. 3 G and H). Together, these data suggest that the GAL4 system most likely produces a higher level of *for* within the MBs than is seen in the *for<sup>R</sup>* flies. In contrast to its effect on olfactory conditioning, expressing *for* using *201y* did not disrupt LTM (Fig. 3 I). Once again, these data indicate that whereas the expression of *for* may appear to provide resilience in one environmental context (sleep deprivation), it may confer an unexpected vulnerability in other situations (LTM).

Finally, we asked whether *for* overexpression in the MBs would phenocopy the *for<sup>R</sup>* response to starvation. Indeed, expressing *for* using either *c739* or *30y* in an otherwise *for<sup>R</sup>* background results in a *for<sup>R</sup>* response to 15 h of starvation beginning 3 h before lights off (Fig. 4 A and B, triangles). However, when *for* is expressed primarily in the  $\gamma$  lobes using the *201y* GAL4 driver, the change in sleep during starvation does not differ from parental controls (Fig. 4 C). Thus, expressing *for*, primarily in the  $\alpha/\beta$  lobes but not the  $\gamma$  lobes, recapitulates many

of the *for<sup>R</sup>* phenotypes. In *for<sup>R</sup>* flies, the increased waking observed during starvation is associated with reduced survival. Thus, we asked whether the increased waking observed in starved *c739* or *30y* flies overexpressing *for* would alter survival. As seen in Fig. 4 D and E, survival was not altered during starvation when *for* was expressed using *c739* or *30y*. However, survival during starvation was increased when using *201y* to express *for* in the  $\gamma$  lobes (Fig. 4 F). Although determining precisely how driving expression of *for* within the  $\gamma$  lobes extends survival is beyond the scope of the current investigation, these data suggest that the MB  $\gamma$  lobes may play a role in controlling and/or responding to metabolic signals. In any event, these data show that the localized expression of *for* within the MBs can alter both short-term and long-term susceptibility to starvation.

## Discussion

Our results not only show that the naturally occurring *foraging* polymorphism modulates sleep homeostasis but also demonstrate that the resistance to sleep loss conferred by higher levels of *foraging* has a tradeoff that is revealed as an increased vulnerability to starvation. In contrast, lower levels of *foraging* are associated with



**Fig. 4.** *for* overexpression in the MBs alters response to starvation. (A–C) *w*;  $for^s;c739/for^s;UAS-for/+$  and *w*;  $for^s;30y/UAS-for$  flies exhibit a *for<sup>s</sup>*-like response to 15 h of starvation, whereas the parental lines retain the *for<sup>s</sup>* phenotype;  $for^s;UAS-for/+$  data are replotted in B and C to facilitate comparisons. In contrast, *w*;  $for^s;201y/for^s;UAS-for/+$  flies and their parental controls (*w*;  $for^s;201y/for^s$ ) exhibit a *for<sup>s</sup>* response to starvation (C). Data are presented as mean  $\pm$  SEM. (D–F) Survival during chronic starvation is not altered when *UAS-for* is expressed in MB  $\alpha$  and  $\beta$  lobes but is increased when *UAS-for* is expressed in MB  $\gamma$  lobes.

resistance to starvation and a corresponding tradeoff, as indicated by an increased vulnerability to SD. Importantly, the phenotypes seen in *foraging* alleles can be largely recapitulated by overexpressing or reducing *foraging* in the  $\alpha/\beta$  lobes of the MBs.

Does the variability in resiliency to sleep loss that is conferred by the *for* polymorphism have ecological relevance? Currently, it is not clear whether the ability to withstand sleep loss can confer an advantage in reproductive fitness and, thus, influence natural selection at the *for* locus. Furthermore, the *for* locus is notably pleiotropic and has been implicated in modulation of learning and memory (8) as well as metabolic plasticity (35), making it difficult to specify which phenotype might respond to a given selection pressure aimed at changing the allelic variation at the *for* locus. It has been established, however, that flies carrying a given *for* allele have a relative fitness advantage when that allele is more rare (4). This finding is consistent with the idea that flies might exploit the resiliencies conferred by their *for* genotype to increase their chances of reproduction. For example, if a rover fly lives in a population where the *for<sup>s</sup>* allele is most frequent, it might increase its reproductive fitness by forgoing sleep to mate at night while its sitter neighbors must rest. This strategy may allow the rover to reduce the competition for a mate yet still maintain optimal functioning the following day. Conversely, a sitter fly might outcompete rover rivals by forgoing a feeding to mate. Under this hypothesis, both natural *for* alleles (along with their associated resiliencies) could be maintained within a given population of flies.

Ecological pressures have been shown to affect cavefish, which have moved from living near the surface of lakes to deeper inside caves (36). Shifting ecological pressures have independently led each of these populations to sleep less than their surface-dwelling ancestors and, importantly, all three have converged upon similar genetic adaptations to adapt to a decrease in sleep time (36). It is possible that the polymorphism in *for* evolved in response to such ecological conditions, such as a prolonged food

shortage that might select for animals able to withstand starvation or to seasonal changes in the length of nights that might place constraints on sleep time.

Ultimately, the extent to which resiliency to sleep loss contributes to the frequency of *for* alleles in clinically varying natural populations of flies remains to be determined. It is important to note, however, that roles for PKG in sleep regulation have been identified in *Caenorhabditis elegans* (7) and in mice (6), indicating that the influence of PKG on sleep regulation is likely to be evolutionarily conserved.

Human studies indicate that individuals vary greatly in their vulnerability to sleep loss (1). With that in mind, several laboratories have begun to examine naturally occurring polymorphisms in humans to determine their role in this differential sensitivity (2, 37, 38). For example, a polymorphism in PERIOD3 (PER3) is associated with larger cognitive deficits following SD (2). Similarly, a functional polymorphism in adenosine deaminase results in increased sleep pressure and increased sensitivity to SD (37). Moreover, a functional polymorphism in brain-derived neurotrophic factor alters EEG slow-wave activity (0.75–4.5 Hz) during both baseline and recovery following SD (38). Pharmaceuticals are commonly used to offset the negative results of SD (e.g., caffeine, Modafinil, etc.). Not surprisingly, polymorphisms also influence the efficacy of drugs to improve performance during SD (39). In this context, the present study suggests that cGMP signaling and PKG are a candidate pathway for sleep resiliency. Single-nucleotide polymorphism in the human *for* ortholog PRKG1 could be investigated for association with sleep loss.

Thus, human studies have begun to identify molecular pathways that alter not only sleep time but resiliency to sleep loss. Unfortunately, determining whether a polymorphism in humans is also associated with unexpected tradeoffs is time-consuming and costly. However, such experiments are tractable in the fly. Indeed, a previous report has found natural genetic variants that contribute

to baseline sleep time during the fly's primary waking period (40). Our data extend these findings to show that naturally occurring polymorphisms alter sleep homeostasis and, importantly, can confer resilience to sleep loss. In addition, our data suggest that the power of *Drosophila* genetics can be applied to these questions to determine the mechanism and extent to which a polymorphism has unexpected tradeoffs. Understanding how these tradeoffs confer resilience or vulnerability to specific environmental challenges is highly relevant for understanding both the importance of sleep during evolution and translational sleep research.

## Materials and Methods

**Flies.** Flies were cultured at 25 °C with 50–60% relative humidity and kept on a diet of yeast, dark corn syrup, and agar under a 12-h light/12-h dark cycle with lights on at 0800 h. Sleep and activity patterns were assessed as described previously (13). Locomotor activity was measured in 1-min bins, and sleep was defined as periods of quiescence lasting at least 5 min.

**Sleep Deprivation.** Four- to seven-day-old females were sleep-deprived for 12 h during the dark phase using the sleep-nullifying apparatus as previously described (13). All sleep and activity were monitored using the TriKinetics *Drosophila* Activity Monitoring System.

**Starvation.** Four- to seven-day-old females were transferred to individual tubes containing a 1% agar gel before lights out and returned to normal fly media the next morning at lights on as previously described (18) or, during survival experiments, kept on agar until death. Cumulative sleep lost and then gained was calculated for the acute starvation experiments by comparing sleep during baseline to the starvation day and two subsequent recovery days.

**APS Short-Term Memory.** One-week-old female flies were placed in a T maze and allowed to choose between a lighted and a dark chamber. Filter paper was wetted with  $10^{-1}$  M quinine hydrochloride solution and placed in the lighted chamber; the percentage of times the fly visits the dark vial was tabulated during 16 trials. The performance index is calculated as the percentage of times the fly chooses the dark vial during the last four trials.

**Courtship Conditioning.** Four- to six-day-old males were trained as previously described (31). The males were exposed to pheromonally feminized *Tai2* males in a spaced training protocol consisting of three 1-h training sessions, each separated by 1 h. Forty-eight hours later, trained and naïve males were exposed to *Tai2* males for a 10-min testing period. The courtship index is defined as the percentage of time that each subject fly spends in courtship behavior during a 10-min testing period.

**Social Enrichment.** Three- to four-day-old flies were divided into a socially isolated group, which were individually housed in 65-mm glass tubes, and a socially enriched group, consisting of 40–45 female flies housed in a single vial as previously described (31). After 5 d of social enrichment/isolation, flies were placed in clean 65-mm glass tubes and sleep was recorded for 3 d. The difference in daytime sleep between isolated and enriched flies was averaged over 3 d and referred to as "ΔSleep."

**Statistics.** All comparisons were done using a Student's *t* test or, if appropriate, ANOVA and subsequent modified Bonferroni tests unless otherwise stated. Statistical tests for data shown in Figs. 1–4 are presented in Tables S2–S5. An asterisk represents  $P < 0.05$  by modified Bonferroni test in all figures unless otherwise described. All statistically different groups are defined as  $P < 0.05$ .

- Van Dongen HPA, Baynard MD, Maislin G, Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: Evidence of trait-like differential vulnerability. *Sleep* 27:423–433.
- Viola AU, et al. (2007) PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol* 17:613–618.
- Osborne KA, et al. (1997) Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* 277:834–836.
- Fitzpatrick MJ, Feder E, Rowe L, Sokolowski MB (2007) Maintaining a behaviour polymorphism by frequency-dependent selection on a single gene. *Nature* 447: 210–212.
- Sokolowski MB (2010) Social interactions in "simple" model systems. *Neuron* 65: 780–794.
- Langmesser S, et al. (2009) cGMP-dependent protein kinase type I is implicated in the regulation of the timing and quality of sleep and wakefulness. *PLoS One* 4:e4238.
- Raizen DM, et al. (2008) Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature* 451:569–572.
- Mery F, Belay AT, So AK-C, Sokolowski MB, Kawecki TJ (2007) Natural polymorphism affecting learning and memory in *Drosophila*. *Proc Natl Acad Sci USA* 104: 13051–13055.
- Papaj DR, Snell-Rood EC (2007) Memory flies sooner from flies that learn faster. *Proc Natl Acad Sci USA* 104:13539–13540.
- Chuah YM, Venkatraman V, Dinges DF, Chee MW (2006) The neural basis of inter-individual variability in inhibitory efficiency after sleep deprivation. *J Neurosci* 26: 7156–7162.
- Graves LA, Heller EA, Pack AI, Abel T (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn Mem* 10(3):168–176.
- Hussaini SA, Bogusch L, Landgraf T, Menzel R (2009) Sleep deprivation affects extinction but not acquisition memory in honeybees. *Learn Mem* 16:698–705.
- Seugnet L, Suzuki Y, Vine L, Gottschalk L, Shaw PJ (2008) D1 receptor activation in the mushroom bodies rescues sleep-loss-induced learning impairments in *Drosophila*. *Curr Biol* 18:1110–1117.
- Seugnet L, et al. (2009) Identifying sleep regulatory genes using a *Drosophila* model of insomnia. *J Neurosci* 29:7148–7157.
- Borbély AA (1977) Sleep in the rat during food deprivation and subsequent restitution of food. *Brain Res* 124:457–471.
- Willie JT, Chemelli RM, Sinton CM, Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci* 24:429–458.
- Keene AC, et al. (2010) Clock and cycle limit starvation-induced sleep loss in *Drosophila*. *Curr Biol* 20:1209–1215.
- Thimgan MS, Suzuki Y, Seugnet L, Gottschalk L, Shaw PJ (2010) The Perilipin homologue, Lipid Storage Droplet 2, regulates sleep homeostasis and prevents learning impairments following sleep loss. *PLoS Biol* 8:e1000466.
- Shaw PJ, Cirelli C, Greenspan RJ, Tononi G (2000) Correlates of sleep and waking in *Drosophila melanogaster*. *Science* 287:1834–1837.
- Thimgan MS, Duntley SP, Shaw PJ (2011) Changes in gene expression with sleep. *J Clin Sleep Med* 7(Suppl 5):S26–S27.
- Seugnet L, Suzuki Y, Donlea JM, Gottschalk L, Shaw PJ (2011) Sleep deprivation during early-adult development results in long-lasting learning deficits in adult *Drosophila*. *Sleep* 34(2):137–146.
- Seugnet L, et al. (2009) Identifying sleep regulatory genes using a *Drosophila* model of insomnia. *J Neurosci* 29:7148–7157.
- Li X, Yu F, Guo A (2009) Sleep deprivation specifically impairs short-term olfactory memory in *Drosophila*. *Sleep* 32:1417–1424.
- Le Bourg E, Buecher C (2002) Learned suppression of photopositive tendencies in *Drosophila melanogaster*. *Anim Learn Behav* 30:330–341.
- Seugnet L, Suzuki Y, Stidd R, Shaw PJ (2009) Aversive phototaxic suppression: Evaluation of a short-term memory assay in *Drosophila melanogaster*. *Genes Brain Behav* 8:377–389.
- Seugnet L, et al. (2011) Notch signaling modulates sleep homeostasis and learning after sleep deprivation in *Drosophila*. *Curr Biol* 21:835–840.
- Walker MP, Stickgold R (2006) Sleep, memory, and plasticity. *Annu Rev Psychol* 57: 139–166.
- van Praag H, Kempermann G, Gage FH (2000) Neural consequences of environmental enrichment. *Nat Rev Neurosci* 1(3):191–198.
- Balling A, Technau GM, Heisenberg M (2009) Are the structural changes in adult *Drosophila* mushroom bodies memory traces? Studies on biochemical learning mutants. *J Neurogenet* 21:209–217.
- Donlea JM, Shaw PJ (2009) Sleeping together: Using social interactions to understand the role of sleep in plasticity. *Adv Genet* 68:57–81.
- Ganguly-Fitzgerald I, Donlea JM, Shaw PJ (2006) Waking experience affects sleep need in *Drosophila*. *Science* 313:1775–1781.
- Donlea JM, Ramanan N, Shaw PJ (2009) Use-dependent plasticity in clock neurons regulates sleep need in *Drosophila*. *Science* 324(5923):105–108.
- McBride SM, et al. (1999) Mushroom body ablation impairs short-term memory and long-term memory of courtship conditioning in *Drosophila melanogaster*. *Neuron* 24: 967–977.
- Joiner WJ, Crocker A, White BH, Sehgal A (2006) Sleep in *Drosophila* is regulated by adult mushroom bodies. *Nature* 441:757–760.
- Kent CF, Daskalchuk T, Cook L, Sokolowski MB, Greenspan RJ (2009) The *Drosophila* foraging gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation. *PLoS Genet* 5:e1000609.
- Duboué ER, Keene AC, Borowsky RL (2011) Evolutionary convergence on sleep loss in cavefish populations. *Curr Biol* 21:671–676.
- Bachmann V, et al. (July 6, 2011) Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. *Cereb Cortex*, 10.1093/cercor/bhr173.
- Bachmann V, et al. (2011) The BDNF Val66Met polymorphism modulates sleep intensity: EEG frequency- and state-specificity. *Sleep*, Available at <http://www.journalsleep.org/acceptedpapers/SP-284-11.pdf>.
- Bodenmann S, et al. (September 26, 2011) Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioral performance and sleep EEG after sleep deprivation. *Br J Pharmacol*, 10.1111/j.1476-5381.2011.01689.x.
- Harbison ST, et al. (2009) Co-regulated transcriptional networks contribute to natural genetic variation in *Drosophila* sleep. *Nat Genet* 41:371–375.