Please cite this article in press as: Kottler et al., A Sleep/Wake Circuit Controls Isoflurane Sensitivity in *Drosophila*, Current Biology (2013), http://dx.doi.org/10.1016/j.cub.2013.02.021

Current Biology *23*, 1–5, April 8, 2013 ©2013 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.02.021

Report

A Sleep/Wake Circuit Controls Isoflurane Sensitivity in *Drosophila*

Benjamin Kottler,[1] Hong Bao,[2] Oressia Zalucki,[1] Wendy Imlach,[1] Michael Troup,[1] Bart van Alphen,[1] Angelique Paulk,[1] Bing Zhang,[2] and Bruno van Swinderen[1,]*
[1]Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia
[2]Department of Biology, University of Oklahoma, Norman, OK 73019, USA

Summary

General anesthesia remains a mysterious phenomenon, even though a number of compelling target proteins and processes have been proposed [1]. General anesthetics such as isoflurane abolish behavioral responsiveness in all animals, and in the mammalian brain, these diverse compounds probably achieve this in part by targeting endogenous sleep mechanisms [2, 3]. However, most animals sleep [4], and they are therefore likely to have conserved sleep processes. A decade of neurogenetic studies of arousal in *Drosophila melanogaster* have identified a number of different neurons and brain structures that modulate sleep duration in the fly brain [5–9], but it has remained unclear until recently whether any neurons might form part of a dedicated circuit that actively controls sleep and wake states in the fly brain, as has been proposed for the mammalian brain [10]. We studied general anesthesia in *Drosophila* by measuring stimulus-induced locomotion under isoflurane gas exposure. Using a syntaxin1A gain-of-function construct, we found that increasing synaptic activity in different *Drosophila* neurons could produce hypersensitivity or resistance to isoflurane. We uncover a common pathway in the fly brain controlling both sleep duration and isoflurane sensitivity, centered on monoaminergic modulation of sleep-promoting neurons of the fan-shaped body.

Results and Discussion

Stimulus-Induced Locomotion Anesthesia Endpoint

Recent *Drosophila* studies have identified sleep- and wake-promoting circuits in the fly brain, converging on the dorsal fan-shaped body of the central complex [9, 11, 12]. Considering the sleep-promoting role that general anesthetics may play on the mammalian brain [2, 3], these findings in *Drosophila* raise the question of whether general anesthesia may also be working in flies through endogenous sleep pathways. If sleep in *Drosophila* is indeed an active brain process, then activation of sleep-promoting neurons in the fly brain should promote general anesthesia, whereas activation of wake-promoting neurons should oppose this process.

Sleep and anesthesia, although describing a similar behavioral endpoint, are measured differently in *Drosophila.* Sleep is typically quantified by inactivity criteria [13, 14], resulting in sleep duration measures over several days and nights (Figure 1A), whereas general anesthesia requires an acute measure of behavioral responsiveness to distressing stimuli under gas exposure. We developed a paradigm to quantify the effect of volatile anesthetic (VA) gas on startle behavior in flies (Figure 1B; see also Supplemental Experimental Procedures available online). Without any VA (0% isoflurane), wild-type flies responded reliably to mechanical vibrations by increased locomotion (measured by velocity) compared to baseline (Figures 1B and S1A–S1D). Adding isoflurane (Figure S1E) abolished stimulus-induced locomotion (Figure S1D), yielding an EC_{50} of ~0.2 vol % for the startle response in wild-type flies (Figure 1B); the same EC_{50} was observed when different batches of flies were assayed at each isoflurane concentration (Figure S1F). The EC_{50} is the VA concentration resulting in half-maximal behavior, which is estimated by logistic regression of the data (see Supplemental Experimental Procedures). By using this highly sensitive isoflurane endpoint (see also [15]), we were able to investigate the circuitry modulating general anesthesia phenotypes in the fly brain and then contrast these to sleep duration effects in transgenic strains.

Increasing Synaptic Release in Different Circuits Produces Resistance and Hypersensitivity to Isoflurane

To investigate how tissue-specific synaptic activity might modulate anesthesia phenotypes, we expressed a mutant form of syntaxin1A ($Syx^{3–69}$) [16] in different brain neurons (Figure 2A), using the UAS-Gal4 gene expression system [17]. Syntaxin1A is a crucial membrane-bound component of the SNARE complex, required for fusing presynaptic vesicles to the neuronal plasma membrane [18]. The $Syx^{3–69}$ mutation decreases the energy required for vesicle fusion with the plasma membrane (Figure 2B) [16], resulting in increased neurotransmitter release, as measured at the larval neuromuscular junction (Figures 2B and 2C).

Increasing synaptic release with UAS-$Syx^{3–69}$ in adult flies across a variety of different neuronal systems and structures yielded significantly different results, either increasing or decreasing isoflurane potency (estimated by the EC_{50}) compared to genetic background controls (Figures 2D and S2). Interestingly, brain-wide expression of UAS-$Syx^{3–69}$ with *elav-Gal4* rendered flies hypersensitive to isoflurane. Expression of UAS-$Syx^{3–69}$ more narrowly in c380 and D42, which express in motor neurons [19], also produced hypersensitivity to isoflurane, whereas expression of UAS-$Syx^{3–69}$ in GABAergic neurons (with the *Gad1*-Gal4 driver [20]) and glutamatergic neurons (with the *vGlut*-Gal4 driver [21]) did not produce any significant hypersensitivity (Figures 2D and S2). Investigation of UAS-$Syx^{3–69}$ effects in the central brain revealed two Gal4 drivers expressing in similar layers of the fan-shaped body (FB) [9] of the central complex (*104y*-Gal4/UAS-$Syx^{3–69}$ and *C5*-Gal4/UAS-$Syx^{3–69}$; see Figure 2E) that produced significant hypersensitivity to isoflurane compared to controls (Figures 2D and S2), whereas drivers expressing in other FB neurons (NP6561 and NP2320) or other brain structures (including the mushroom bodies) had no effect. In contrast, UAS-$Syx^{3–69}$ expression in monoamine-expressing neurons (dopamine and octopamine/tyramine) produced isoflurane resistance (Figures 2C and S2). That these effects

*Correspondence: b.vanswinderen@uq.edu.au

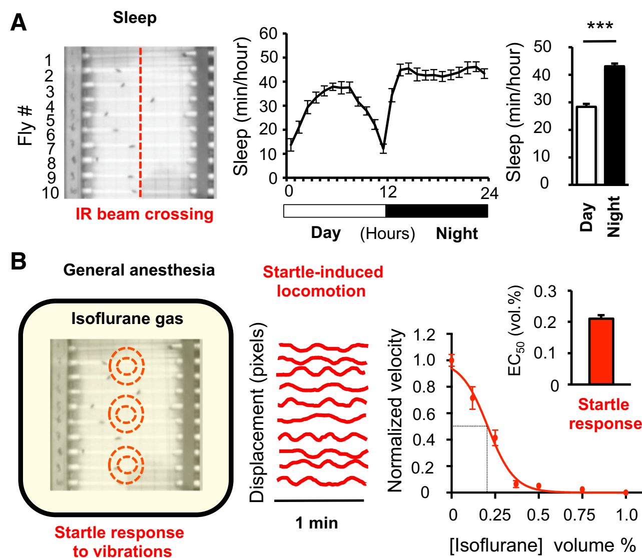

Figure 1. Sleep Duration and General Anesthesia

(A) Left panel: schema of sleep setup. Individual fly locomotion across the midline (dashed line) is tallied by infrared beam interruptions over several days; epochs without any crossings for 5 min or longer are counted as sleep. Middle panel: average sleep (min/hr ± SEM) for wild-type (CS) females (n = 32). Right panel: summary data for day and night sleep (***$p < 0.001$, by t test comparing means).

(B) Left panel: schema of anesthesia apparatus. Flies housed individually in glass tubes respond to vibration stimuli from small motors attached to the scaffold (represented by dashed circles) by increased locomotion. The arousal-testing device is housed in a 1 l chamber that can be equilibrated to different concentrations of anesthetic gas. Middle panel: flies are filmed and automatically tracked in order to quantify a locomotion profile per fly (y axis represents horizontal displacement along each tube; x axis represents time). Right panel: nonlinear regression of normalized stimulus-induced velocity ± SEM, under increasing isoflurane concentrations (vol % atm). The EC_{50} is the isoflurane concentration resulting in half-maximal behavior (dotted lines). Inset: estimated EC_{50} ± standard error of the estimate (SEE) for stimulus-induced locomotion in wild-type females (n = 20). See Figure S1 for additional information on the assay.

might be restricted to the fly central nervous system was supported by the lack of any effect in *Tdc1*-Gal4/UAS-*Syx*$^{3–69}$ (Figure S3A), which drives expression of octopamine/tyramine outside the brain [22]. Increasing synaptic release simultaneously from dopaminergic, octopaminergic, and tyraminergic neurons in *Tdc2*-Gal4;*Th*-Gal4/UAS-*Syx*$^{3–69}$ flies did not produce greater isoflurane resistance than that shown by either Gal4 driver individually (Figure S3B), suggesting a convergence of effects rather than additive effects. Similarly, increasing synaptic release simultaneously from dopaminergic and serotonergic neurons in *Ddc*-Gal4/UAS-*Syx*$^{3–69}$ flies produced isoflurane resistance that was not greater than that shown by either the *Th* or *Trh*-Gal4 driver individually (Figure S3C).

To confirm our resistance phenotypes, we expressed another transgenic construct, UAS-*Vr1*, which activates neurons by acute feeding of capsaicin to flies (Figure 3A, left panel) [9], in *Th* and *Tdc2* neurons. Consistent with our preceding results, activating dopaminergic and octopaminergic/tyraminergic neurons by feeding capsaicin to *Th*-Gal4/UAS-*Vr1* and *Tdc2*-Gal4/UAS-*Vr1* adult flies conferred resistance to isoflurane in both strains, compared to sham-fed controls (Figures 3A, S3D, and S3E). By comparison, similarly treated *Gad1*-Gal4/UAS-*Vr1* flies or UAS-*Vr1*/+ controls showed no significant effect (Figures 3A, S3F, and S3G).

Dopaminergic Modulation of Sleep-Promoting Neurons Produces Resistance to Isoflurane

The wake-promoting role of dopamine (DA) in *Drosophila* is well established [23]. To further confirm the relevance of dopamine to isoflurane sensitivity, we investigated two mutants impacting dopamine function. *dumb*^{2} and *fumin* are two mutants that affect dopaminergic signaling in opposite ways. *dumb*^{2} is a hypomorphic allele of the dDA1 receptor (DopR) resulting in lower dopamine levels [24], whereas *fumin* is a mutant for the dopamine transporter gene (dDAT) leading to an increase in dopamine at the synapse [25]. These mutations have opposite effects on sleep duration [12]. We found that the mutants also have opposite effects on isoflurane sensitivity: *fumin* is resistant compared to genetic background controls, whereas *dumb*^{2} is hypersensitive (Figures 3B, S3H, and S3I).

Two recent studies identified specific DA neurons that innervate the dorsal fan-shaped body (dFB) to modulate sleep duration in *Drosophila* [11, 12]. We found in our UAS-*Syx*$^{3–69}$ screen (Figure 2D) that two sleep-inducing Gal4 lines that express in the FB (104y and C5; Figure 2E) are hypersensitive to isoflurane. Therefore, one simple model potentially explaining our anesthesia results is that activation of the 104y and C5-Gal4 neurons in the FB decreases behavioral responsiveness under anesthesia (consistent with their sleep-promoting role), whereas DA activity inhibits these neurons (in line with DA's wake-promoting role). Consistent with this model, DA innervates the same FB layers that have been suggested to modulate arousal in 104y and C5-Gal4 strains (Figure 2E) [11], DopR is highly expressed in the FB [26], and DopR has been shown to inhibit neurotransmission in *Drosophila* cell cultures [27]. To test whether DA modulation of sleep-promoting neurons controls behavioral responsiveness under general anesthesia, we targeted the expression of DopR in the 104y neurons specifically. RNAi-mediated knockdown of DopR in these neurons produced flies that are hypersensitive to isoflurane, resembling the *dumb*^{2} mutant (Figures 3B and S3J). Combining *fumin* and *dumb*^{2}, in the *DAT*^{fmn};*DA1*^{dumb2} double mutant, abolished *fumin*-induced resistance (Figures 3B and S3K), confirming the role of this specific DA receptor in the anesthesia phenotype. Because the *dumb*^{2} mutation harbors a UAS construct [12], we could express wild-type DopR in different neurons under Gal4 control. We found that expression of DopR in the sleep-promoting neurons (104y-Gal4) was sufficient to rescue *fumin*-induced resistance in the double-mutant background (Figures 3B and S3K). In contrast, DopR expression in the mushroom bodies (using OK107-Gal4 [12]) did not rescue *fumin*-induced resistance (Figures 3B and S3K). These results confirm the sufficiency of the sleep-promoting system encompassed by 104y for producing isoflurane resistance via DopR.

Sleep-Promoting Neurons Regulate General Anesthesia Phenotypes

Our data so far suggest that increased dopaminergic modulation of sleep-promoting (104y-Gal4) neurons via DopR results in isoflurane resistance, and conversely that decreased DA modulation of sleep-promoting neurons leads to isoflurane hypersensitivity. This model therefore proposes that the sleep-promoting neurons are downstream of modulatory dopaminergic neurons, and that it is synaptic output from

Please cite this article in press as: Kottler et al., A Sleep/Wake Circuit Controls Isoflurane Sensitivity in *Drosophila*, Current Biology (2013), http://dx.doi.org/10.1016/j.cub.2013.02.021

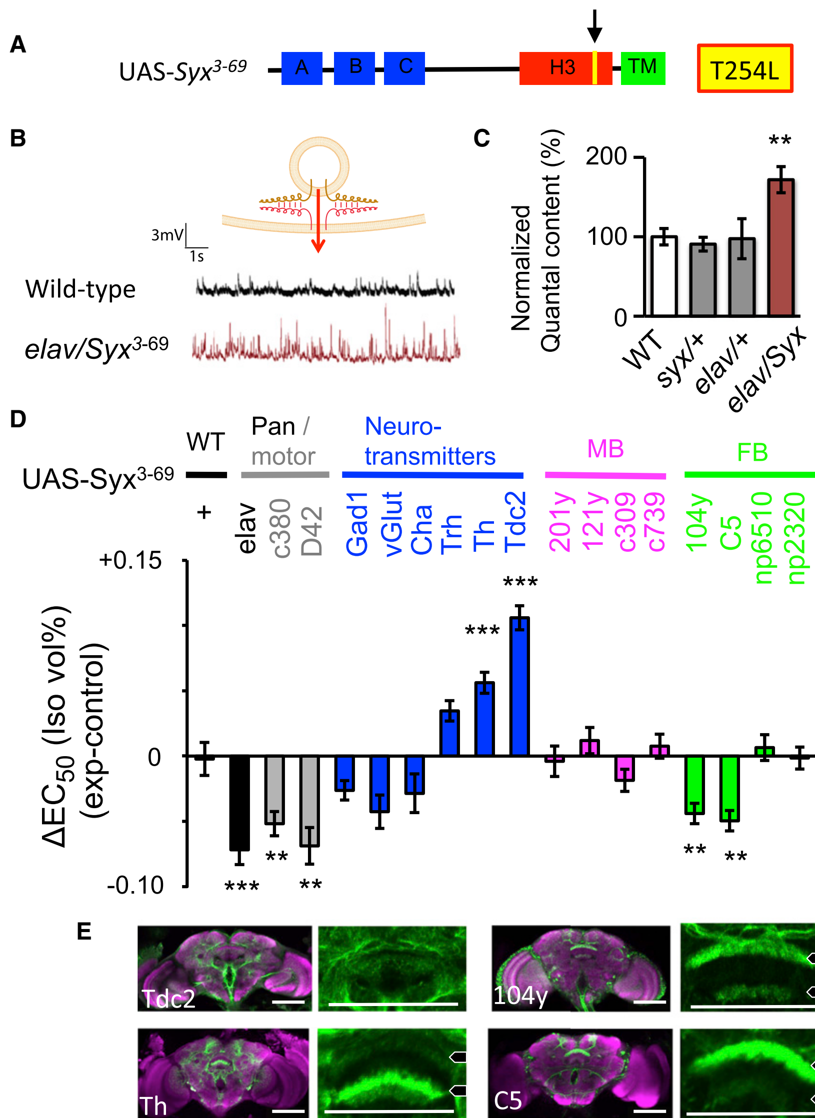

Figure 2. Effects of Increasing Synaptic Release

(A) UAS-*Syx*$^{3-69}$ construct, indicating the position of the T254L mutation in the H3 domain of the molecule. TM, transmembrane domain; A, B, C, N-terminal interaction domains.

(B) Schema showing vesicle fusion (syntaxin1A molecule in red) and sample traces of postsynaptic potentials measured at the larval neuromuscular junction (NMJ) in wild-type and *elav*-Gal4/UAS-*Syx*$^{3-69}$ animals.

(C) Estimation of neurotransmitter release (normalized quantal content ± SEM) at the larval NMJ for *elav*-Gal4/UAS-*Syx*$^{3-69}$ and control strains (see Supplemental Experimental Procedures for additional data). **p < 0.01 by ANOVA adjusted for multiple comparisons; n = 8 for each strain. WT, wild-type.

(D) ΔEC_{50} ± SEE (isoflurane vol % atm) for each Gal4 line indicated when combined with UAS-*Syx*$^{3-69}$ and tested as adults. ΔEC_{50} is calculated by subtracting the control EC_{50} (Gal4/+) from the experimental EC_{50} (Gal4/UAS-*Syx*$^{3-69}$). Each strain was compared to its genetic control, represented by the zero line on the y axis, by simultaneous curve-fitting statistics. See Figure S2 for all dose-response curves. p values were adjusted for 18 planned comparisons: *p < 0.05, **p < 0.01, ***p < 0.001, calculated by extra sum-of-squares F test between estimated EC_{50} of experimental and control strains; n > 30 flies per group (see Figure S2 for exact n and p values). Wild-type (CS) was compared to CS/UAS-*Syx*$^{3-69}$. Pan/motor, panneural and motor-neuron expressing; MB, mushroom body expressing; FB, fan-shaped body expressing.

(E) UAS-GFP expression (green) driven by Tdc2-Gal4 (top left), Th-Gal4 (bottom left), 104y-Gal4 (top right), and C5-Gal4 (bottom right). A focus on the FB region is shown for each Gal4, with dorsal and medial FB layers indicated (arrowheads), with different levels of intensity as evident by GFP expression. Magenta is nc82 staining. Scale bar represents 100 μm for all.

sleep-promoting neurons specifically that regulates anesthesia phenotypes. To test this, we expressed *Syx*$^{3-69}$ simultaneously in sleep-promoting (104y-Gal4) and wake-promoting (Th-Gal4) neurons. These flies (104y-Gal4;*Th*-Gal4/UAS-*Syx*$^{3-69}$) are hypersensitive to isoflurane (Figures 3B and S3L), suggesting that synaptic output from 104y-Gal4 neurons overrides DA effects on isoflurane sensitivity. We also found significant isoflurane hypersensitivity by using a different way of increasing neuronal excitability in sleep-promoting neurons [9], in 104y-Gal4/UAS-*NachBAC* flies (Figure S3M). Our synaptic manipulation of 104y neurons now narrows the anesthesia effect (and sleep effect; Figure S4) to their synaptic output.

We therefore next investigated what the consequence on general anesthesia would be if synaptic output from sleep-promoting neurons were blocked. If increased synaptic release from 104y neurons produces isoflurane hypersensitivity (Figures 2D and 3B), then would decreased synaptic release from these neurons produce isoflurane resistance? To test this, we blocked synaptic output from sleep-promoting neurons using a constitutive tetanus toxin construct, UAS-tnt, which inactivates the synaptic release machinery [28]. This manipulation proved lethal at the pupal stage (data not shown), so we expressed tetanus toxin acutely in adult flies by using a temperature-sensitive repressor of Gal4, *tubulin*-Gal80^{TS} [29] (Figure 3C, left panel). Blocking synaptic release from the 104y neurons in adults produced isoflurane resistance, compared to controls where synaptic release was not blocked (Figures 3C and S3N). Significant resistance was also produced by blocking synaptic release from C5-Gal4 neurons (Figures 3C and S3O), which have overlapping expression with 104y-Gal4 in the FB (Figure 2E), indicating that the FB is likely to be involved in modulating the anesthesia phenotype. Acutely blocking synaptic release from these sleep-promoting neurons also significantly decreased daytime sleep duration (Figures S4J and S4K).

Isoflurane Anesthesia and Sleep Are Negatively Correlated

The opposing effects that FB activation and monoaminergic activation have on anesthetic sensitivity mirror the opposing effect that these neurons have on sleep duration. To determine the extent to which sleep and general anesthesia phenotypes are correlated in the fly brain, we measured sleep duration for strains that yielded significantly different isoflurane sensitivities. These included various UAS/Gal4 lines and mutants describing the DA-FB pathway uncovered in this study, as well as their relevant control strains (Figure S4). As shown in Figure 4, we found a strong negative correlation (r = −0.81,

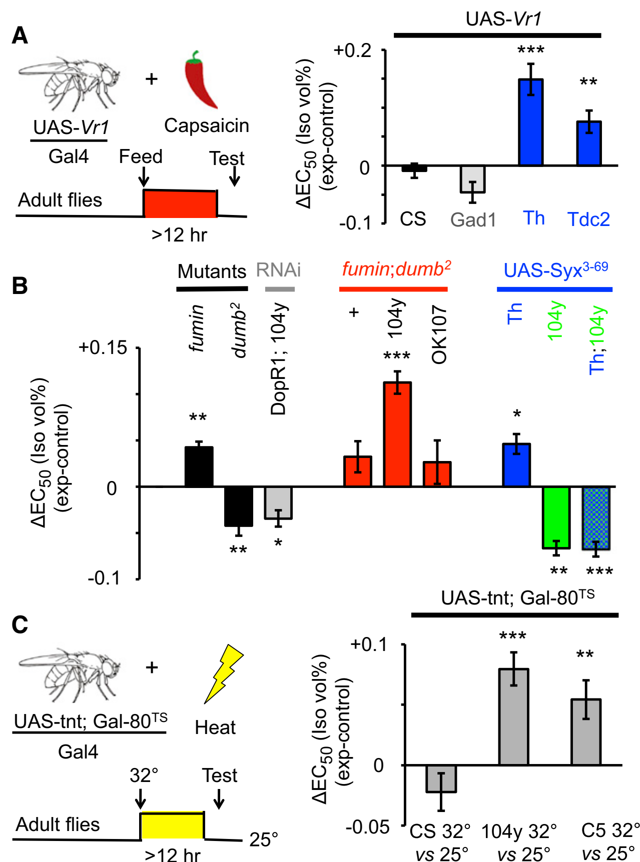

Figure 3. Acute Resistance Effects and Mutants

(A) Left: adult UAS-*Vr1*/Gal4 flies were fed 100 mM capsaicin in food to activate specific Gal4 neurons. Flies were tested for isoflurane sensitivity after >12 hr capsaicin exposure. Right: ΔEC_{50} ± SEE for four strains crossed to UAS-*Vr1* following capsaicin exposure, compared to sham-fed controls (represented by the zero y axis line). p values were adjusted for four comparisons: **p < 0.01, ***p < 0.001, calculated by extra sum-of-squares F test between estimated EC_{50} of experimental and control strains; n > 30 flies per group (see Figure S3 for exact n and p values and all dose-response curves).

(B) ΔEC_{50} ± SEE for select mutants, UAS-RNAi knockdown, and Gal4 doubles. Each strain is compared to its genetic control strain (represented by the zero y axis line). p values were adjusted for three comparisons per set of three: **p < 0.01, *p < 0.05, calculated by extra sum-of-squares F test between estimated EC_{50} of experimental and control strains; n > 30 (see Figure S3 for exact n and p values and all dose-response curves).

(C) Left: exposing adult UAS-tnt;Gal80^{TS}/Gal4 flies to heat (32°C) acutely blocks synaptic release in specified Gal4 neurons. Flies were tested for isoflurane sensitivity at room temperature (25°C) after >12 hr heat. Right: targeting synaptic release in sleep-promoting neurons (104y- and C5-Gal4) in adults produces isoflurane resistance compared to unheated controls (represented by the zero y axis line). p values were adjusted for three comparisons: **p < 0.01, ***p < 0.001, calculated by extra sum-of-squares F test between estimated EC_{50} of experimental and control strains; n = 30 for all experiments (see Figure S3 for exact n and p values and all dose-response curves).

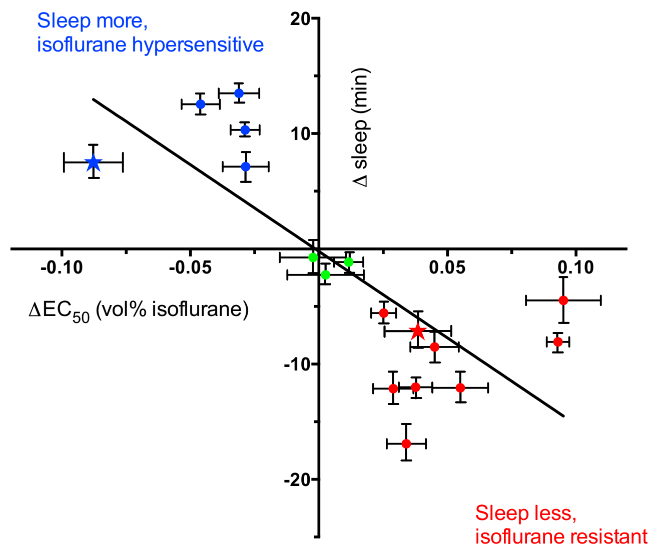

Figure 4. Contrasting Sleep Duration and Isoflurane EC_{50} Data

Scatterplot of ΔEC_{50} ± SEE (isoflurane vol % atm) against change in total daytime sleep duration (Δmin/hr ± SEM) for data from Figures 2 and 3. A linear fit is drawn through the data; r = −0.81 (p < 0.0001). Blue star: sleep-deprived flies; red star: methamphetamine-fed flies. Blue dots: 104y/UAS-*Syx*$^{3-69}$, C5/UAS-*Syx*$^{3-69}$, *dumb*^{2}, 104y/UAS-NaChBac; green dots: CS, Tdc1/UAS-*Syx*$^{3-69}$, CS/UAS-tnt;Gal80^{TS} (32°C); red dots: Th/UAS-*Syx*$^{3-69}$, Tdc2/UAS-*Syx*$^{3-69}$, Trh/UAS-*Syx*$^{3-69}$, Ddc/UAS-*Syx*$^{3-69}$, Tdc2+Th/UAS-*Syx*$^{3-69}$, 104y/UAS-tnt;Gal80^{TS} (32°C), C5/UAS-tnt;Gal80^{TS} (32°C), *fumin*. All isoflurane dose-response curves are in Figures S2 and S3; all corresponding sleep profiles are in Figure S4.

p = 0.0001) between daytime sleep duration and isoflurane sensitivity: resistant flies slept less, and hypersensitive flies slept more. This tight relationship between daytime sleep and anesthesia phenotypes was also evident for night-time sleep (r = −0.64, p = 0.001), and the correlation extended beyond mutant strains to behavioral and pharmacological manipulations: sleep-deprived flies slept more and were hypersensitive to isoflurane, whereas methamphetamine-fed flies slept less and were resistant to isoflurane (Figure 4, blue and red stars, respectively; Figures S3 and S4).

The negative correlation between isoflurane EC_{50} and sleep duration that we have uncovered in *Drosophila* provides support for a potential overlap between endogenous sleep mechanisms and general anesthesia in the fly brain. This conclusion is consistent with an earlier study showing that a short-sleeping mutant in *Drosophila*, *shaker*, is also resistant to the general anesthetics isoflurane and sevoflurane [30]. It is therefore possible that the *shaker* potassium channel is involved in regulating neuronal activity levels in the DA-FB circuit to control both sleep and anesthesia phenotypes. In mammals, general anesthetics appear to achieve a sedative effect by activating sleep-promoting neurons in the hypothalamus [3]. Whether simpler animals also become anesthetized by activation of sleep-promoting neurons seems to first depend on whether those animals really sleep. Our study confirms the importance of the DA-FB pathway in the fly brain for *Drosophila* arousal in general, and our results predict that the sleep-promoting neurons (e.g., 104y cells) should be activated under general anesthesia as well as natural sleep.

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.021.

Acknowledgments

We thank Kazuhiko Kume for genetic reagents and Rudolf Bohm for assistance with generating the UAS-*Syx*$^{3-69}$ construct. This work was supported

Please cite this article in press as: Kottler et al., A Sleep/Wake Circuit Controls Isoflurane Sensitivity in *Drosophila*, Current Biology (2013), http://dx.doi.org/10.1016/j.cub.2013.02.021

by an Australian Research Council Discovery project grant (DP1093968) and Future Fellowship (FT100100725) to B.v.S. and by grants from the National Science Foundation (NSF; IOS-0822236 and IOS-1025556) to B.Z.

Received: November 22, 2012
Revised: January 7, 2013
Accepted: February 8, 2013
Published: March 14, 2013

References

1. Franks, N.P. (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat. Rev. Neurosci. *9*, 370–386.
2. Nelson, L.E., Guo, T.Z., Lu, J., Saper, C.B., Franks, N.P., and Maze, M. (2002). The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat. Neurosci. *5*, 979–984.
3. Moore, J.T., Chen, J., Han, B., Meng, Q.C., Veasey, S.C., Beck, S.G., and Kelz, M.B. (2012). Direct activation of sleep-promoting vlpo neurons by volatile anesthetics contributes to anesthetic hypnosis. Curr. Biol. *22*, 2008–2016.
4. Cirelli, C., and Tononi, G. (2008). Is sleep essential? PLoS Biol. *6*, e216.
5. Joiner, W.J., Crocker, A., White, B.H., and Sehgal, A. (2006). Sleep in Drosophila is regulated by adult mushroom bodies. Nature *441*, 757–760.
6. Pitman, J.L., McGill, J.J., Keegan, K.P., and Allada, R. (2006). A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature *441*, 753–756.
7. Foltenyi, K., Greenspan, R.J., and Newport, J.W. (2007). Activation of EGFR and ERK by rhomboid signaling regulates the consolidation and maintenance of sleep in Drosophila. Nat. Neurosci. *10*, 1160–1167.
8. Chung, B.Y., Kilman, V.L., Keath, J.R., Pitman, J.L., and Allada, R. (2009). The GABA(A) receptor RDL acts in peptidergic PDF neurons to promote sleep in Drosophila. Curr. Biol. *19*, 386–390.
9. Donlea, J.M., Thimgan, M.S., Suzuki, Y., Gottschalk, L., and Shaw, P.J. (2011). Inducing sleep by remote control facilitates memory consolidation in Drosophila. Science *332*, 1571–1576.
10. Saper, C.B., Chou, T.C., and Scammell, T.E. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci. *24*, 726–731.
11. Liu, Q., Liu, S., Kodama, L., Driscoll, M.R., and Wu, M.N. (2012). Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. Curr. Biol. *22*, 2114–2123.
12. Ueno, T., Tomita, J., Tanimoto, H., Endo, K., Ito, K., Kume, S., and Kume, K. (2012). Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. Nat. Neurosci. *15*, 1516–1523.
13. Campbell, S.S., and Tobler, I. (1984). Animal sleep: a review of sleep duration across phylogeny. Neurosci. Biobehav. Rev. *8*, 269–300.
14. Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. Science *287*, 1834–1837.
15. van Swinderen, B. (2006). A succession of anesthetic endpoints in the Drosophila brain. J. Neurobiol. *66*, 1195–1211.
16. Lagow, R.D., Bao, H., Cohen, E.N., Daniels, R.W., Zuzek, A., Williams, W.H., Macleod, G.T., Sutton, R.B., and Zhang, B. (2007). Modification of a hydrophobic layer by a point mutation in syntaxin 1A regulates the rate of synaptic vesicle fusion. PLoS Biol. *5*, e72.
17. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
18. Wu, M.N., Fergestad, T., Lloyd, T.E., He, Y., Broadie, K., and Bellen, H.J. (1999). Syntaxin 1A interacts with multiple exocytic proteins to regulate neurotransmitter release in vivo. Neuron *23*, 593–605.
19. Parkes, T.L., Elia, A.J., Dickinson, D., Hilliker, A.J., Phillips, J.P., and Boulianne, G.L. (1998). Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nat. Genet. *19*, 171–174.
20. Küppers, B., Sánchez-Soriano, N., Letzkus, J., Technau, G.M., and Prokop, A. (2003). In developing Drosophila neurones the production of gamma-amino butyric acid is tightly regulated downstream of glutamate decarboxylase translation and can be influenced by calcium. J. Neurochem. *84*, 939–951.
21. Kolodziejczyk, A., Sun, X., Meinertzhagen, I.A., and Nässel, D.R. (2008). Glutamate, GABA and acetylcholine signaling components in the lamina of the Drosophila visual system. PLoS ONE *3*, e2110.
22. Cole, S.H., Carney, G.E., McClung, C.A., Willard, S.S., Taylor, B.J., and Hirsh, J. (2005). Two functional but noncomplementing Drosophila tyrosine decarboxylase genes: distinct roles for neural tyramine and octopamine in female fertility. J. Biol. Chem. *280*, 14948–14955.
23. Van Swinderen, B., and Andretic, R. (2011). Dopamine in Drosophila: setting arousal thresholds in a miniature brain. Proc. Biol. Sci. *278*, 906–913.
24. Kim, Y.C., Lee, H.G., and Han, K.A. (2007). D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in Drosophila. J. Neurosci. *27*, 7640–7647.
25. Kume, K., Kume, S., Park, S.K., Hirsh, J., and Jackson, F.R. (2005). Dopamine is a regulator of arousal in the fruit fly. J. Neurosci. *25*, 7377–7384.
26. Lee, H.G., Seong, C.S., Kim, Y.C., Davis, R.L., and Han, K.A. (2003). Octopamine receptor OAMB is required for ovulation in Drosophila melanogaster. Dev. Biol. *264*, 179–190.
27. Yuan, N., and Lee, D. (2007). Suppression of excitatory cholinergic synaptic transmission by Drosophila dopamine D1-like receptors. Eur. J. Neurosci. *26*, 2417–2427.
28. Sweeney, S.T., Broadie, K., Keane, J., Niemann, H., and O'Kane, C.J. (1995). Targeted expression of tetanus toxin light chain in Drosophila specifically eliminates synaptic transmission and causes behavioral defects. Neuron *14*, 341–351.
29. McGuire, S.E., Mao, Z., and Davis, R.L. (2004). Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. Sci. STKE *2004*, pl6.
30. Weber, B., Schaper, C., Bushey, D., Rohlfs, M., Steinfath, M., Tononi, G., Cirelli, C., Scholz, J., and Bein, B. (2009). Increased volatile anesthetic requirement in short-sleeping Drosophila mutants. Anesthesiology *110*, 313–316.