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Use-Dependent Plasticity in Clock Neurons Regulates Sleep Need in *Drosophila*

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Abstract

Sleep is important for memory consolidation and is responsive to waking experience. Clock circuitry is uniquely positioned to coordinate interactions between processes underlying memory and sleep need. Flies increase sleep both after exposure to an enriched social environment and after protocols that induce long-term memory. We found that flies mutant for *rutabaga*, *period*, and *blistered* were deficient for experience-dependent increases in sleep. Rescue of each of these genes within the ventral lateral neurons (LN_Vs) restores increased sleep after social enrichment. Social experiences that induce increased sleep were associated with an increase in the number of synaptic terminals in the LN_V projections into the medulla. The number of synaptic terminals was reduced during sleep and this decline was prevented by sleep deprivation.

Although sleep is a process that is necessary for survival, the functions of sleep are unknown (1,2). Sleep is regulated by circadian influences and is important for consolidation of long-term memory (LTM) (3–5). Additionally, LTM is modulated by circadian mechanisms (6,7). Because the relationship between sleep, memory, and circadian rhythms seem to be phylogenetically conserved, *Drosophila* can be used to explain mechanisms that coordinate these processes. *Drosophila* show an increase in daytime sleep after exposure to socially enriched environments (5). Similarly, an increase in sleep after courtship conditioning is necessary for LTM (5).

Increased sleep after social enrichment is dependent upon genes that are required for learning and memory, including genes that alter cyclic adenosine monophosphate signaling (5). Although newly eclosed flies that are mutant for the adenylyl cyclase *rutabaga* (*rut*²⁰⁸⁰) show increased sleep after social enrichment, 3- 4-day-old adult *rut* mutants do not respond to changes in the social environment (fig. S1). Elevating wild-type *rut* in adult flies with an RU486-inducible driver rescued experience-dependent increases in sleep in adult *rut* mutants (fig. S1); vehicle-treated siblings showed no increase in sleep (fig. S1). To identify circuits that mediate experience-dependent increases in sleep, we used a series of GAL4 lines to drive wild-type *rut* expression in brain circuits (Fig. 1A). Figure S2 illustrates the data analysis used to quantify each GAL4 rescue. Expression of UAS-*rut* using *pdf*-GAL4 restored the increase in daytime sleep and daytime sleep-bout duration, although to a lesser extent than GSeIav (Fig. 1, B to E). The expression pattern of *pdf*-GAL4 is limited to the ventral lateral neurons (LN_Vs), a group of clock neurons that express *pigment-dispersing factor* (*pdf*) (8,9). Although *pdf* is the only known output from the LN_Vs, flies mutant for *pdf* show a wild-type increase in sleep (fig. S3).

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Given this role of clock cells, we examined the clock gene *period* (*per*), which is expressed in the LN_Vs and is required for LTM (6). Rescue of wild-type *per* using a 7.2-kb fragment of the *per* genomic sequence (*per*⁰¹; *per*+7.2-2) restored expression of PER at CT0 within the LN_Vs as well as the dorsal lateral neurons, LN_Ds (Fig. 1F); mutant flies carrying a null mutation, *per*⁰¹, expressed no PER (Fig. 1G). Although *per*⁰¹ mutants showed no increase in sleep after social enrichment, *per*⁰¹; *per*+7.2-2 flies displayed normal experience-dependent increases in sleep (Fig. 1H). *per*⁰¹ mutants have no LTM when tested 48 hours after training and only show a transient increase in sleep (Fig. 1, I and J). *per*⁰¹; *per*+7.2-2 flies displayed LTM (Fig. 1K) and increases in sleep (Fig. 1, L and M). Although *per* levels are low in mutants for *Clock* and *cycle*, both acquire LTM (6) and increase sleep after social enrichment (5). Thus, only a very small amount of *per* may be required to support increased sleep and LTM.

To further investigate the role of synaptic plasticity in clock cells, we used the *Drosophila* homolog for serum response factor (*SRF*), *blistered* (*bs*). In mice, *SRF* is essential for activity-induced gene expression and plays an important role in synaptic long-term potentiation (10) and in contextual habituation (11). *bs* retains a 93% identity with *SRF* within the DNA-binding MCM1-ARG80-Agamous-Deficiens-SRF (MADS) domain (12). Social enrichment elevated the transcription of *bs* in wild-type *Canton-S* (*Cs*) flies (Fig. 2A). Mutants carrying a P element inserted into the *bs* gene (P{GAL4}*bs*¹³⁴⁸) do not increase sleep after social enrichment (Fig. 2B). This deficit was also found in flies carrying either of two other mutant alleles for *bs* (*bs*² and *bs*³) and was present in flies that are homozygous for mutant *bs* alleles and flies that have been outcrossed to either *Cs* or to flies carrying the In(2LR)*Px*⁴ deficiency (Fig. 2C). The P-element insertion in *bs*¹³⁴⁸ preserves the MADS domain; similar N-terminal truncated mutant *SRF* acts as dominant negative (13). *BS* is expressed throughout the brain, including *pdf*-expressing LN_Vs (Fig. 2, D to F). When UAS-*egfp* was driven by P{GAL4}*bs*¹³⁴⁸, expression was restricted to a small number of neurons, including the LN_Vs (Fig. 2, G and I). Expression of *bs* using P{GAL4}*bs*¹³⁴⁸ to drive either of two wild-type *bs* (UAS-*bs*) constructs rescued experience-dependent increases in sleep (Fig. 2J). Moreover, inducing *bs* expression within the LN_Vs using *pdf*-GAL4 increased sleep after social enrichment (Fig. 2K).

To establish whether expression of *bs* is required for LTM, we tested flies carrying the P{GAL4}*bs*¹³⁴⁸ mutant allele using courtship conditioning. Although P{GAL4}*bs*¹³⁴⁸/+ flies acquire short-term memory (Fig. 2L), LTM was impaired (Fig. 2M, left). Rescue of wild-type *bs* using P{GAL4}*bs*¹³⁴⁸ restored LTM (Fig. 2M, right). Next, we used the GAL4 repressor *cry*-GAL80 to block UAS-*bs* expression within the LNs. Although UAS-*bs*/+; *cry*-gal80/+ control flies showed significant courtship suppression (Fig. 2N, left), P{GAL4}*bs*¹³⁴⁸/UAS-*bs*; *cry*-GAL80/+ flies had no LTM (Fig. 2N, right), which suggests a role for the LNs, although we cannot exclude a role for the dorsal neurons (DNs). Although *SRF* deletion in mouse forebrain results in neurons with abnormal morphology (14), the morphology of LN_Vs in mutant P{GAL4}*bs*¹³⁴⁸/+ flies (fig. S4A) did not differ from that of LN_Vs in P{GAL4}*bs*¹³⁴⁸/UAS-*bs* rescue flies (fig. S4B). All three mutants for *bs* had intact circadian rhythms and showed anticipatory activity before light-dark transitions; only *bs*³ flies show an altered period under constant darkness (fig. S5, A to F). These findings suggest that there are no developmental abnormalities in the LN_Vs *bs* mutants.

Hypomorphic alleles for *bs* prevent proper wing development through interactions with *Epidermal growth factor receptor* (*Egfr*) signaling (15–17). Because *Egfr* alters sleep in *Drosophila* (18), interactions between *bs* and *Egfr* may regulate responses to social experience. After social enrichment, transcription of *Egfr* was significantly elevated in *Cs* flies (Fig. 3A). The *Egfr* genomic sequence contains several CC(A/T)6GG CArG elements that can be bound by *bs* to promote transcription (Fig. 3B), and transcription of *Egfr* was

significantly reduced in *bs* mutants (data not shown). Thus, we used P{GAL4}*bs*¹³⁴⁸ to drive expression of a constitutively active *Egfr* construct (UAS-*Egfr**). Although P{GAL4}*bs*¹³⁴⁸/+ mutants showed no change in sleep after social enrichment (Fig. 3C), activation of *Egfr* in P{GAL4}*bs*¹³⁴⁸/+;UAS-*Egfr**/+ flies increased sleep (Fig. 3, D and E). Conversely, the expression of a dominant-negative construct for *Egfr* (UAS-*Egfr*^{DN}) using *pdf*-GAL4 prevented increases in sleep after social enrichment, but parental controls (*pdf*-GAL4/+ and UAS-*Egfr*^{DN}/+) were wild-type (Fig. 3F).

A recent theory proposes that a function of sleep is to downscale synaptic connections (19). Moreover, structural plasticity can be induced by environmental manipulation in *Drosophila* (20). To quantify the effect of social enrichment on the number of post-synaptic terminals in LN_V projections, we used *pdf*-GAL4 to drive expression of a green fluorescent protein (GFP)-tagged construct of the postsynaptic protein discs-large (UAS-*dlg*WT-gfp). After 5 days of social enrichment, LN_V projections into the medulla of *pdf*-GAL4/+;UAS-*dlg*WT-gfp/+ flies contained significantly more GFP-positive terminals (Fig. 4, A to C). Although we have not demonstrated that the labeled synaptic terminals are functional, these tools have been used to quantify synapses (21). The expression of the UAS-*dlg*WT-GFP marker did not alter synaptic function in a wild-type background (20) and did not prevent the increase in sleep when expressed using *pdf*-GAL4 after social enrichment (fig. S6). To determine the effect of waking on synapse number, socially isolated *pdf*-GAL4/+;UAS-*dlg*WT-gfp/+ flies and their enriched siblings either were allowed to sleep ad libitum or were sleep deprived for 48 hours after social enrichment. Although the number of *dlg*-GFP positive terminals remained elevated in sleep-deprived socially enriched flies, terminal number was significantly reduced in siblings that were allowed to sleep (Fig. 4D). Similarly, the number of presynaptic terminals in LN_V projections into the medulla using a GFP-tagged construct of the presynaptic protein synaptobrevin (UAS-*VAMP*-GFP) in *pdf*-GAL4/+;UAS-*VAMP*-GFP/+ flies was increased (Fig. 4, E to G). After 48 hours of recovery, socially enriched *pdf*-GAL4/+;UAS-*VAMP*-GFP/+ flies had a reduced number of *VAMP*-GFP-positive presynaptic terminals relative to their sleep-deprived siblings (Fig. 4H). A recent study has reported a clock-dependent remodeling in the axonal terminals of the PDF circuit that is highest during the day (22). Recent data indicates that hyperexcitation of a subset of the LN_Vs suppresses sleep in *Drosophila* (23–25). Together with our results, these data suggest that the PDF circuit is well suited to test the hypothesis that sleep acts to downscale synaptic connections that are potentiated during waking experience.

Supplementary Material

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References and Notes

1. Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB. Science 1983;221:182. [PubMed: 6857280]
2. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Nature 2002;417:287. [PubMed: 12015603]
3. Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Neuron 2002;35:205. [PubMed: 12123620]
4. Graves LA, Heller EA, Pack AI, Abel T. Learn. Mem 2003;10:168. [PubMed: 12773581]
5. Ganguly-Fitzgerald I, Donlea J, Shaw PJ. Science 2006;313:1775. [PubMed: 16990546]

6. Sakai T, Tamura T, Kitamoto T, Kidokoro Y. *Proc. Natl. Acad. Sci. U.S.A* 2004;101:16058. [PubMed: 15522971]
7. Decker S, McConnaughey S, Page TL. *Proc. Natl. Acad. Sci. U.S.A* 2007;104:15905. [PubMed: 17893338]
8. Helfrich-Forster C, Homberg U. *J. Comp. Neurol* 1993;337:177. [PubMed: 8276996]
9. Renn SC, Park JH, Rosbash M, Hall JC, Taghert PH. *Cell* 1999;99:791. [PubMed: 10619432]
10. Ramanan N, et al. *Nat. Neurosci* 2005;8:759. [PubMed: 15880109]
11. Etkin A, et al. *Neuron* 2006;50:127. [PubMed: 16600861]
12. Affolter M, et al. *Development* 1994;120:743. [PubMed: 7600954]
13. Gauthier-Rouviere C, et al. *Exp. Cell Res* 1993;209:208. [PubMed: 8262137]
14. Knoll B, et al. *Nat. Neurosci* 2006;9:195. [PubMed: 16415869]
15. Fristrom D, et al. *Development* 1994;120:2661. [PubMed: 7956840]
16. Montagne J, et al. *Development* 1996;122:2589. [PubMed: 8787734]
17. Roch F, Baonza A, Martin-Blanco E, Garcia-Bellido A. *Development* 1998;125:1823. [PubMed: 9550715]
18. Foltenyi K, Greenspan RJ, Newport JW. *Nat. Neurosci* 2007;10:1160. [PubMed: 17694052]
19. Tononi G, Cirelli C. *Brain Res. Bull* 2003;62:143. [PubMed: 14638388]
20. Barth M, Heisenberg M. *Learn. Mem* 1997;4:219. [PubMed: 10456065]
21. Zhang Y, et al. *Neuron* 2007;53:201. [PubMed: 17224403]
22. Fernández MP, Berni J, Ceriani MF. *PLoS Biol* 2008;6:e69. [PubMed: 18366255]
23. Sheeba V, et al. *Curr. Biol* 2008;18:1537. [PubMed: 18771923]
24. Parisky KM, et al. *Neuron* 2008;60:672. [PubMed: 19038223]
25. Shang Y, Griffith LC, Rosbash M. *Proc. Natl. Acad. Sci. U.S.A* 2008;105:19587. [PubMed: 19060186]

Fig. 1.

Clock cells regulate experience-dependent increases in sleep. **(A)** Data for each GAL4 line ($GAL4>+;rut^{2080}/+;UAS-rut/+$) is compared to its parental line ($GAL4>+;rut^{2080}/+$). No increase in Δ daytime sleep is observed in the absence of GAL4 [$(rut^{2080}/+;UAS-rut/+)$ ($rut^{2080}/+$); white bar]. The mean Δ daytime sleep for *Gs-elav*-GAL4 (RU+ versus RU-) is shown to facilitate comparisons (black). One-way analysis of variance (ANOVA) for genotype, $F_{(33,903)} = 9.09$ (*, $P < .05$ with correction for 34 comparisons; $n \geq 16$ for all groups). **(B and C)** Sleep after social enrichment in mutant $rut^{2080}/+;pdf-GAL4/+$ flies and $rut^{2080}/+;pdf-GAL4/+;UAS-rut/+$ flies ($n = 16$ in each group). **(D and E)** Average daytime sleep and sleep-bout duration in $rut^{2080}/+;pdf-GAL4/+$ mutant and $rut^{2080}/+;pdf-GAL4/+;UAS-rut/+$ rescue flies. Two-way ANOVA reveals a genotype by condition interaction for sleep [$F_{(1,57)} = 4.44$, $P = 0.03$] and bouts [$F_{(1,57)} = 6.59$, $P = 0.013$] (*, $P < .05$, planned pairwise comparisons with a Tukey correction; $n = 14$ to 16 for all groups). **(F and G)** PER immunohistochemistry of $per^{01};per+7.2-2$ and per^{01} mutants. **(H)** Δ daytime sleep in per^{01} mutants and $per^{01};per+7.2-2$ flies ($P = 0.0002$; $n = 30$ to 32 in each group). **(I)** Male per^{01} mutants do not exhibit a reduction in courtship 48 hours after a spaced courtship conditioning (N, Naïve; T, Trained; $n = 13$ to 14). **(J)** per^{01} flies only exhibit a transient increase in sleep immediately after training. **(K)** $per^{01};per+7.2-2$ flies exhibit suppression of courtship 48 hours after training ($P = 0.001$; $n = 13$ in each group). **(L)** $per^{01};per+7.2-2$ males also show sustained increases in sleep for 2 days. **(M)** Δ daytime sleep is quantified for per^{01} and $per^{01};per+7.2-2$ males. Two-way ANOVA reveals a genotype by day interaction $F_{(1,39)} = 7.48$, $P = 0.009$ (*, $P < .05$ planned pairwise comparisons with a Tukey correction; $n = 13$ to 14 each group).

Fig. 2.

blistered regulates experience-dependent increases in sleep. (A) *bs* transcripts are significantly elevated in socially enriched *Cs* flies compared with their isolated siblings ($P = 0.03$). (B) Flies homozygous for the $P\{GAL4\}bs^{1348}$ insertion do not respond to social enrichment with an increase in sleep ($n = 16$ in each group). (C) Mean Δ daytime sleep is absent in bs^2 and bs^3 mutants and persists when each allele (bs^2 , bs^3 , or $P\{GAL4\}bs^{1348}$) is outcrossed to *Cs* or with the Deficiency $In(2LR)Px^4$. One-way ANOVA for genotype [$F_{(9,124)} = 2.73$; $P = 0.04$; $n = 32$ in each group]. (D) Expression of UAS-*egfp* using *pdf*-GAL4 labels the cell bodies of LN_V s. (E) Immunohistochemistry using mouse antibody to BS (diluted 1:1000). (F) Colocalization of BS and *pdf*-GAL4 indicates that BS is expressed in *pdf*-expressing LN_V s. (G) $P\{GAL4\}bs^{1348}$ was used to drive UAS-*egfp*, and the expression pattern was evaluated using confocal microscopy. (H) Brains of $P\{GAL4\}bs^{1348}/+;UAS-egfp$ flies were colabeled with guinea pig antibody to PDF (diluted 1:10,000) (I) Colocalization of GFP with PDF indicates that $P\{GAL4\}bs^{1348}$ drives GAL4 expression in *PDF*-expressing LN_V s (arrows). (J) The failure to respond to social enrichment with an increase in sleep can be rescued by combining $P\{GAL4\}bs^{1348}$ with either of two separate UAS-*bs* alleles. One-way ANOVA for genotype [$F_{(2,45)} = 22.86$; $P = 1.4 \times 10^{-5}$; $P < .05$ Bonferroni post-hoc test; $n = 16$ in each group]. (K) Expressing wild-type *bs* using *pdf*-GAL4 in an otherwise *bs* mutant background (*pdf*-GAL4/+; bs^2/b^3 ;UAS-*bs*) rescues increases in Δ daytime sleep; parental lines are shown in white. The lower Δ sleep versus 2J may indicate a partial rescue. One-way ANOVA for genotype [$F_{(2,45)} = 6.30$; $P = 0.003$; $P < .05$, Bonferroni post-hoc test; $n = 16$ in each group]. (L) Courtship suppression in $P\{GAL4\}bs^{1348}$ mutants tested 5 min after Courtship Conditioning White bars represent naïve males; dark bars represent trained males ($P = 0.02$; $n = 13$ in each group). (M) LTM is disrupted in $P\{GAL4\}bs^{1348}/+$ mutants tested 48 hours after training and rescued by expression of UAS-*bs*. Two-way ANOVA reveals a genotype by condition interaction [$F_{(2,72)} = 16.73$; $P = 0.0001$] ($P < .05$, planned pairwise comparisons with a Tukey correction; $n = 13$ in each group). (N) Blocking GAL4 expression in clock cells using

cry-GAL80 prevents rescue of LTM. Genotype by condition interaction [$F_{(1,48)} = 2.32$; $P = 0.14$] (*, $P < .05$, planned pairwise comparisons with a Tukey correction; $n = 13$ in each group).

Fig. 3.

Egfr mediates experience-dependent sleep. (A) Transcription of *Egfr* is significantly elevated in socially enriched *Cs* flies compared with their isolated siblings, as measured by quantitative polymerase chain reaction. (B) Genomic *Egfr* sequence contains several SRF-binding CArG elements. (C and D) Driving the expression of UAS-*Egfr*⁺ with *P{GAL4}bs¹³⁴⁸* restores ΔSleep after social enrichment. (E) Summary of the response for data shown in (C) and (D) (*, $P = 7 \times 10^{-5}$; $n = 16$ in each group). (F) No change in Δ sleep is observed in *pdf-GAL4/UAS-Egfr^{DN}*. One-way ANOVA for genotype $F_{(2,44)} = 7.75$, $P = 0.001$ (*, $P < .05$, Bonferroni correction; $n = 15$ to 16 in each group).

Fig. 4.

LN_V synapse number in medulla. (**A** and **B**) LN_V projections in socially enriched *pdf-GAL4/+;UAS-dlgWT-gfp/+* flies contain more GFP-positive terminals than their socially isolated siblings when evaluated using confocal microscopy. (**C**) Relative quantification of *dlg*-GFP-immunopositive terminals in socially isolated *pdf-GAL4/+;UAS-dlgWT-gfp/+* flies versus socially enriched siblings ($P = 1.5 \times 10^{-5}$; $n = 33$ to 34 in each group). (**D**) *dlg*-GFP positive terminals 48 hours after social enrichment in sleep-deprived flies and their normally sleeping siblings. ($P = 0.003$; $n = 15$ to 18 in each group). (**E** and **F**) Social enrichment of *pdf-GAL4/+;UAS-VAMP-GFP/+* flies induces a modest increase of LN_V terminals relative to isolated siblings. (**G**) Relative quantification of *VAMP*-GFP-positive terminals in socially isolated *pdf-GAL4/+;UAS-VAMP-gfp/+* flies and their socially enriched siblings ($P = 0.03$; $n = 16$ to 18 in each group). (**H**) *UAS-VAMP-gfp*-positive terminals 48 hours after social enrichment in sleep-deprived flies and their normally sleeping siblings ($P = 0.01$; $n = 18$ in each group).