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Spatiotemporal Rescue of Memory Dysfunction in *Drosophila*

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We have developed a method for temporal and regional gene expression targeting (TARGET) in *Drosophila* and show the simultaneous spatial and temporal rescue of a memory defect. The transient expression of the *rutabaga*-encoded adenylyl cyclase in the mushroom bodies of the adult brain was necessary and sufficient to rescue the *rutabaga* memory deficit, which rules out a developmental brain defect in the etiology of this deficit and demonstrates an acute role for *rutabaga* in memory formation in these neurons. The TARGET system offers general utility in simultaneously addressing issues of when and where gene products are required.

Memory traces are typically thought to manifest as plastic changes in neuronal physiology that occur in specific regions of the brain. In *Drosophila*, distinct brain structures known as the mushroom bodies have been demonstrated to have an important role in the associative learning and memory of olfactory stimuli (*1*–*6*); however, the location of olfactory memory acquisition and storage in the *Drosophila* brain has remained unknown. One strategy for identifying the brain regions in which plasticity is required for the establishment of memory traces is to target the expression of a gene thought to be required for neuronal plasticity to specific regions of the brain in an animal mutant for that gene, using the GAL4/UAS system (*6*, *7*).

However, a critical question when using this approach is whether memory rescue represents the rescue of a physiological defect in the neurons themselves or of a defect in the proper development of the animal's nervous system. The expression of the *rutabaga*-encoded type I adenylyl cyclase in the mushroom bodies during development and adulthood is sufficient to rescue the short-term memory defect in *rutabaga* mutant flies, suggesting that the memory trace might localize to the mushroom bodies (*6*). The GAL4 elements that were used in these experiments, however, drive expression in both the developing and adult mushroom bodies and, therefore, did not distinguish between a developmental and adult requirement for *rutabaga* for memory rescue. The ability to distinguish between a developmental defect in neuronal connectivity and a physiological defect in neuronal plasticity is crucial for understanding the implications of behavioral rescue experiments for the localization of a memory trace.

This distinction is particularly relevant in the case of type I adenylyl cyclases. Type I adenylyl cyclases are required for some forms of synaptic plasticity and memory (*8*). As a class, they respond synergistically to intracellular calcium and heterotrimeric GTP-binding protein signaling, thus representing a potential molecular substrate for the detection and integration of the coincidence between a conditioned stimulus and an unconditioned stimulus on a neuron (*9*–*11*). However, the type I adenylyl cyclases also have well-known roles in neural development. The mouse mutant *barrelless*, which lacks barrels in the primary somatosensory cortex and exhibits defects in the patterning of this brain region in the early postnatal stage (*12*), results from a mutation in type I adenylyl cyclase (*13*). In *Drosophila*, defects in the development of the mushroom bodies have been observed in *rutabaga* mutants, including a possible reduction in the number of axonal tracts (*14*), a reduction in the volume of their dendritic arbors (*15*), and defects in the structural plasticity of the mushroom bodies in response to developmental conditions (*16*). These observations suggest that *rutabaga* is potentially required for the normal development of the mushroom bodies.

We developed a technology that allows for both temporal and regional gene expression targeting (TARGET) in *Drosophila* using the conventional GAL4/UAS system (Fig. 1A) (*17*). The traditional GAL4/UAS system provides tight regional control of gene expression, but it lacks experimenter-defined temporal control and, hence, does not enable a systematic study of the critical time periods in which the expression of a transgene is required in a specific tissue for a particular phenotype. We therefore cloned a temperature-sensitive version of the GAL80 protein (GAL80ts), which normally functions as a repressor of GAL4 in yeast, and tested it for temperature-sensitive regulation of GAL4 activity in both yeast and *Drosophila* (*18*).

We first tested the ability of GAL80ts to repress GAL4-induced expression in the brains of adult flies. Fig. 1B, panels *i* and *ii*, demonstrates the fluorescence pattern of green fluorescent protein expression in the brains of adult flies that carry the GAL4 driver c739, which drives expression at high levels in the α and β lobes of the mushroom bodies (*3*, *19*). Flies were raised at 19°C and were either untreated (–hs, *i*) or treated (+hs, *ii*) with a 12-hour exposure to 30°C. In the absence of heat shock, flies that also expressed GAL80ts from the tubulin 1α promoter showed undetectable levels of GFP fluorescence in the brain (Fig. 1B, *iii*), whereas flies treated with the heat pulse regimen showed increased levels of GFP fluorescence in the mushroom bodies (Fig. 1B, *iv*). Flies expressing wild-type GAL80 from the tubulin 1α promoter showed no GFP fluorescence in either the untreated or treated groups (Fig. 1B, *v* and *vi*). Flies raised at 25°C carrying $GAL4^{c739}$ along with GAL80ts and the UAS-GFP reporter showed faint but detectable levels of GFP fluorescence.

We next measured GFP mRNA levels by reverse transcription–polymerase chain reaction (RT-PCR) in flies after their exposure to varying lengths of heat shock. Expression of GFP was detected as early as 30 min into the heat shock. After 3 hours at 32°C, the GFP

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expression reached half-maximal levels in flies carrying GAL80ts compared to GFP expression in control flies without GAL80ts (the + group). After 6 hours at 32°C, the levels of GFP mRNA were equivalent to those of control flies that did not carry the GAL80ts transgene. Fig. 1C demonstrates the off-rate kinetics of the system after a 6-hour heat shock at 32°C. After heat shock, flies were allowed to recover for varying lengths of time at 19°C and subsequently assayed for GFP expression by RT-PCR. Expression levels of GFP fell to half-maximal levels between 12 and 18 hours after the temperature downshift. By 36 hours after heat shock, GFP expression levels were similar to those in control flies that never received heat shock.

We then sought to use the TARGET system to drive the expression of *rutabaga* in both time and space, in order to delineate the temporal requirements for *rutabaga* expression in the mushroom bodies during development and adulthood that are necessary for rescue of the associated memory defect. As an initial control experiment, we first demonstrated that the mushroom body GAL4 drivers c772 and 247, in combination with a UAS-*rutabaga* transgene, could provide rescue of the *rutabaga* memory phenotype when the flies were reared throughout development and maintained as adults at 19°C. Both the c772 and 247 drivers provided strong rescue of the memory phenotype under these conditions (Fig. 2A), demonstrating that the GAL4 activity in flies maintained at 19°C is sufficient for behavioral rescue in combination with a UAS-*rutabaga* transgene.

We next asked whether GAL80ts could, under permissive conditions, repress the rescue effects shown in Fig. 2A. Fig. 2, B and C, shows the results obtained when flies that bore either the 247 or c772 driver, in combination with UAS-*rutabaga* in the presence of GAL80ts, were reared and maintained at 19°C throughout the experiment. Flies of the genotype rut^{2080};GAL80ts;247/UAS-*rutabaga* had performance levels indistinguishable from those of control flies that were rut^{2080};GAL80ts;247 alone (Fig. 2B). Flies of the genotype rut^{2080};c772;GAL80ts/UAS-*rutabaga* also performed similarly to control flies of the genotype rut^{2080};c772;GAL80ts that lacked the UAS-*rutabaga* transgene (Fig. 2C), demonstrating that GAL80ts can repress the rescue effect under conditions that are permissive for activity of GAL80ts.

Next, we sought to determine whether we could observe the rescue effect in the presence of GAL80ts if flies were reared and maintained as adults at 30°C, a temperature restrictive for the function of GAL80ts. The memory performance of flies that carry the GAL4 driver in combination with GAL80ts and the UAS-*rutabaga* transgene was indistinguishable from that of control flies that are wild-type at the *rutabaga* locus (Fig. 2, D and E), demonstrating that the inactivation of GAL80ts function at 30°C throughout development and adulthood results in behavioral rescue similar to that shown in Fig. 2A.

Because the GAL4 drivers 247 and c772 drive expression in the mushroom bodies during development and throughout adulthood (*20*), we wondered whether the rescue effects seen in Fig. 2, D and E, were due to the expression of the UAS-*rutabaga* construct during development, during adulthood, or during both stages. Because adult flies are competent to show maximal memory performance as early as 1 day after eclosion, we reasoned that any critical period for the development of the mushroom body must occur before this point. We therefore reared flies at 30°C throughout development and the first day after emergence from the pupal case, and then transferred them to 19°C for 7 days in order to repress the expression of the UAS-*rutabaga* transgene. Kinetic experiments measuring mRNA levels indicated that the off-rate kinetics for the TARGET system occurred with a half-time of approximately 15 hours (Fig. 1C, bottom). Under these conditions, flies of the genotype rut^{2080};GAL80ts;247/UAS-*rutabaga* performed at the same level as control flies that were rut^{2080};GAL80ts;247, and these two lines performed significantly lower than control flies of the genotype CS;GAL80ts;247, which carried a wild-type Canton S (CS) X chromosome (Fig. 2F). Flies of the genotype rut^{2080};c772;GAL80ts/UAS-*rutabaga* also performed

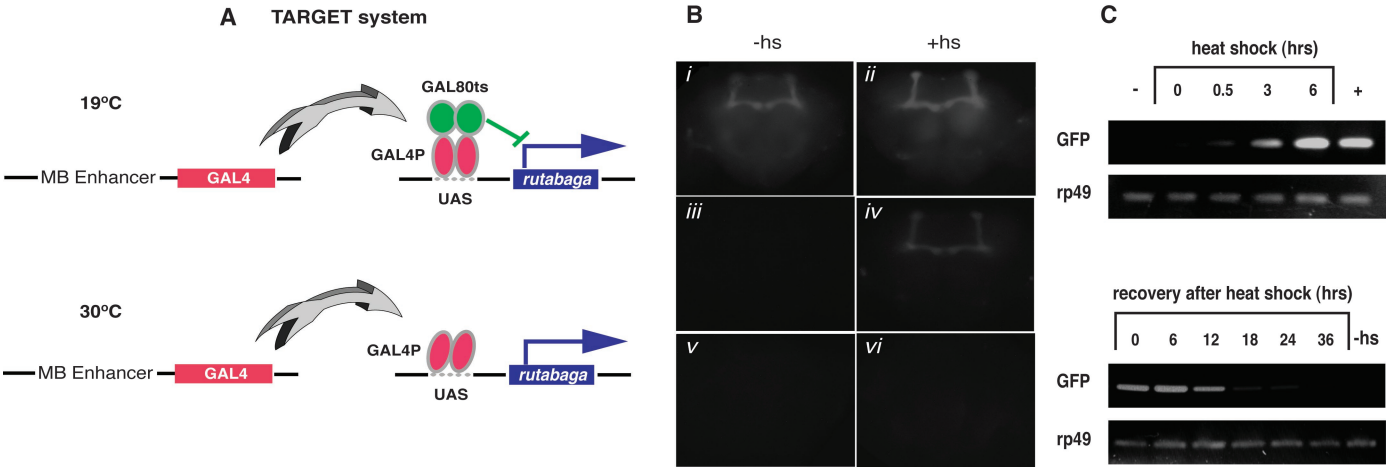

Fig. 1. The TARGET system. (**A**) In the conventional GAL4/UAS system (*7*) a P element carrying the GAL4 coding region drives the expression of GAL4 protein in a specific tissue on the basis of proximity of the P element to a tissue-specific enhancer. GAL4 protein (GAL4P) then binds to its cognate UAS binding site and activates transcription of the downstream effector gene. In the TARGET system, a temperature-sensitive GAL80 protein (GAL80ts), expressed ubiquitously from the tubulin 1α promoter, represses the transcriptional activity of GAL4 at 19°C and thus prevents the expression of the UAS-*rutabaga* transgene, but becomes inactive at 30°C, allowing GAL4 to drive the expression of the UAS-*rutabaga* transgene in the mushroom bodies. MB, mushroom body. (**B**) GFP fluorescent images of dissected brains from adult flies raised at 19°C and either untreated (*i*, *iii*, and *v*) or treated (*ii*, *iv*, and *vi*) with a 12-hour heat treatment at 30°C. hs, heat shock Genotypes: GAL4c^{739}; UAS-GFP (*i* and *ii*), GAL4c^{739}; UAS-GFP;tubP-GAL80ts (*iii* and *iv*), and GAL4c^{739};UAS-GFP;tubP-GAL80 (*v* and *vi*). (**C**) Kinetics of GAL4-mediated transcription after heat shock. Top: RT-PCR for GFP expression was performed immediately after 0 hours, 0.5 hours, 3 hours, and 6 hours of heat shock at 32°C for the genotype GAL4c^{155},UAS-mCD8-GFP/+;tubP-GAL80ts/+. Control genotypes included w(CS10), a Canton S strain carrying a mutation in the *white* gene (–, left) and GAL4c^{155},UAS-mCD8-GFP/+, which carries the mouse CD8-GFP fusion gene (+, right). Bottom: RT-PCR for GFP expression was performed at 0 hours, 6 hours, 12 hours, 18 hours, 24 hours, and 36 hours after a 6-hour heat shock at 32°C in flies of the genotype GAL4c^{155},UAS-mCD8-GFP/+;tubP-GAL80ts/+. The negative control in this experiment (–) consisted of flies of the same genotype that had not received heat shock. In both experiments, the constitutively expressed ribosomal protein gene 49 (rp 49) rp49 served as an internal control for the PCR amplification.

indistinguishably from control flies that were rut^{2080};c772;GAL80ts, and both of these lines performed significantly lower than flies of the genotype CS;c772;GAL80ts (Fig. 2G).

We next sought to determine whether expression of *rutabaga* exclusively during the adult phase was sufficient to demonstrate rescue of the memory defect. Flies were raised at 19°C until 1 day after eclosion and then shifted to 30°C. To approximate the length of heat treatment in the developmental group (Fig. 2, F and G), flies were maintained at 30°C for 6 days as adults. For flies of the genotypes rut^{2080};GAL80ts;247/UAS-*rutabaga* and rut^{2080};c772;GAL80ts/UAS-*rutabaga*, expression of *rutabaga* specifically in the adult phase was sufficient to demonstrate significant rescue of the memory phenotype compared to flies that lacked the UAS-*rutabaga*

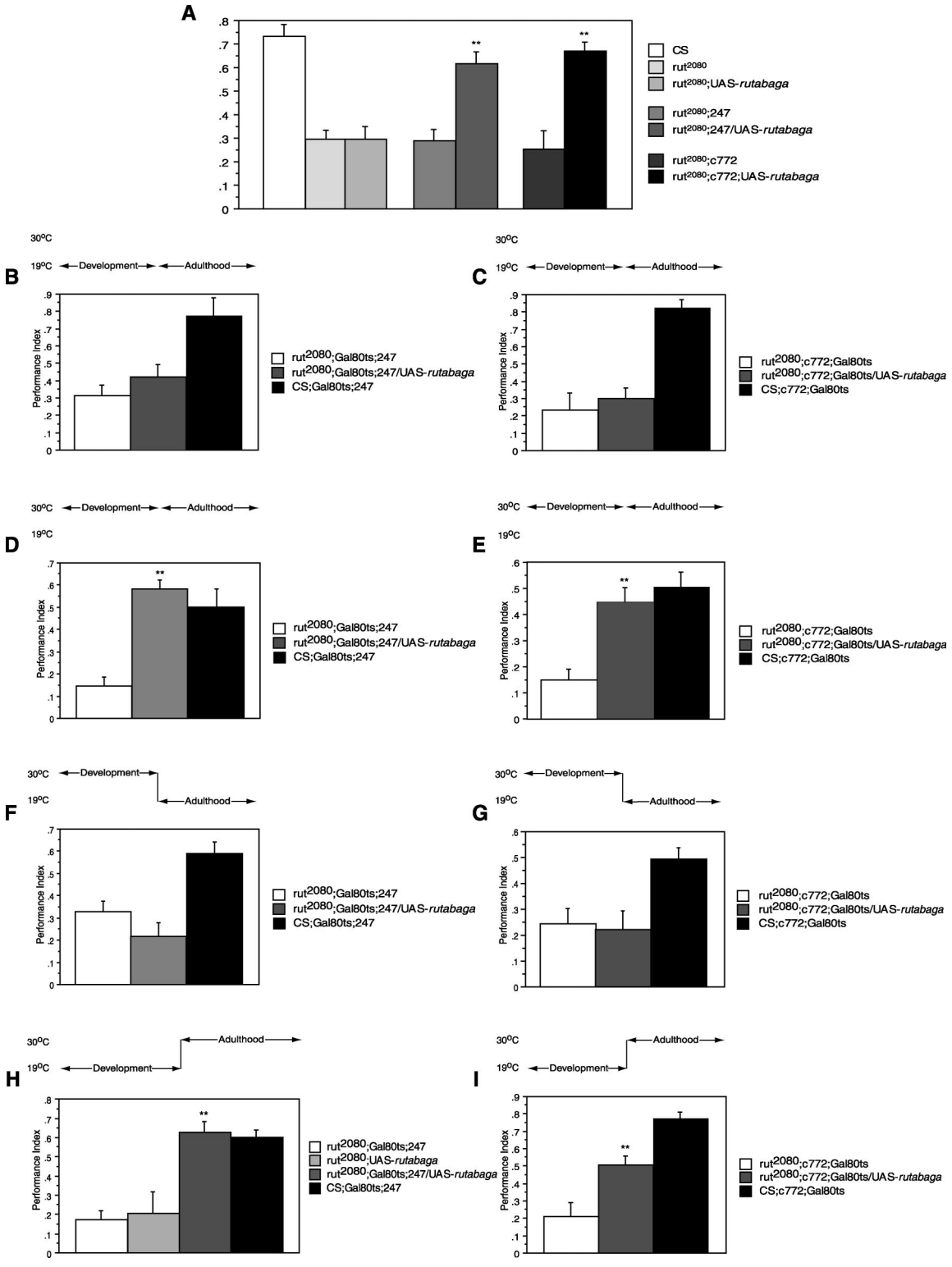

Fig. 2. Adult expression of *rutabaga* is necessary and sufficient for memory rescue. In all experiments, the Performance indicies were subjected to a one-way analysis of variance with genotype as the main effect, followed by post-hoc analysis with the Student-Neuman-Keuls test. Asterisks indicate a significance difference from the mutant group. (**A**) Flies bearing the GAL4 drivers in combination with the UAS-*rutabaga* transgene demonstrated significant rescue of memory performance over mutant control groups when reared at 19°C. (**B** and **C**) GAL80ts repressed the GAL4/UAS *rutabaga* rescue under permissive conditions. (**D** and **E**) Rescue of the *rutabaga* memory phenotype by expression of *rutabaga* throughout development and adulthood. Flies were reared and maintained as adults at 30°C. (**F** and **G**) *Rutabaga* expression during development was not sufficient for memory rescue in adults. Flies were reared throughout development at 30°C and shifted to 19°C 1 day after eclosion. Flies were then maintained at 19°C for 7 days until training. (**H** and **I**) *Rutabaga* expression in the mushroom bodies during adulthood was both necessary and sufficient for memory rescue. Flies were reared throughout development at 19°C and shifted to 30°C 1 day after eclosion. Flies were then maintained at 30°C for 6 days, at which point they were trained and tested.

transgene (Fig. 2, H and I). To examine whether shorter periods of heat exposure were sufficient to achieve rescue, we exposed flies of the genotype rut^{2080};c772; GAL80ts/UAS-*rutabaga* to 12-hour heat shock (*21*). These flies performed significantly better than control flies that lacked the UAS-*rutabaga* transgene [performance index = 0.373 ± 0.06 versus 0.172 ± 0.06, $P < 0.02$].

To confirm that the expression of *rutabaga* in the mushroom bodies correlated with our behavioral results, we examined the levels of Rutabaga protein in the mushroom bodies of flies subjected to the different treatments demonstrated in Fig. 2. Figure 3A demonstrates by immunohistochemistry low background levels of Rutabaga staining in the mushroom bodies of control flies of the genotype rut^{2080};UAS-*rutabaga*. Fig. 3B demonstrates strong Rutabaga staining in the mushroom bodies of flies of the genotype rut^{2080};c772;UAS-*rutabaga*, which carry the GAL4 driver/UAS-*rutabaga* combination and show normal memory performance levels (Fig. 2A). Fig. 3C demonstrates low background levels of Rutabaga staining in the mushroom bodies of flies of the genotype rut^{2080};c772; GAL80ts/UAS-*rutabaga* when the flies were reared and maintained at 19°C throughout adulthood, conditions under which behavioral rescue was repressed (Fig. 2, B, and C). Fig. 3D shows high levels of Rutabaga staining in the mushroom bodies of flies of the same genotype as in Fig. 3C, when the flies were reared and maintained at 30°C throughout development and adulthood, conditions under which behavioral rescue was observed (Fig. 2, D and E). When flies of the same genotype were raised at 30°C throughout development and shifted to 19°C for 7 days as adults, expression levels of Rutabaga in the mushroom bodies fell back to basal levels (Fig. 3E). In contrast, when the flies were raised at 19°C throughout development and shifted to 30°C for 6 days as adults (Fig. 3F), the levels of Rutabaga protein in the mushroom bodies were distinctly elevated above the background levels shown in Fig. 3, A, C, and E.

By using the TARGET system to drive the expression of a UAS-*rutabaga* construct in the mushroom bodies during specific temporal phases, we have demonstrated the spatial and temporal rescue of a memory defect. *Rutabaga* expression in the mushroom bodies of the adult fly is both necessary and sufficient to correct the olfactory memory defect in *rutabaga* mutant flies, which rules out a need for *rutabaga* expression during the development of the mushroom bodies in order to observe behavioral rescue. The demonstration of an acute role for the *rutabaga*-encoded type I adenylyl cyclase within the adult mushroom body neurons provides direct evidence in support of models that envision the *rutabaga*-encoded type I adenylyl cyclase serving as a molecular coincidence detector for the convergence of simultaneous signals on a neuron. In this capacity, it represents a potential molecular mechanism for the acquisition of a memory trace through the detection and integration of the coincidence between a conditioned stimulus and an unconditioned stimulus upon a set of neurons.

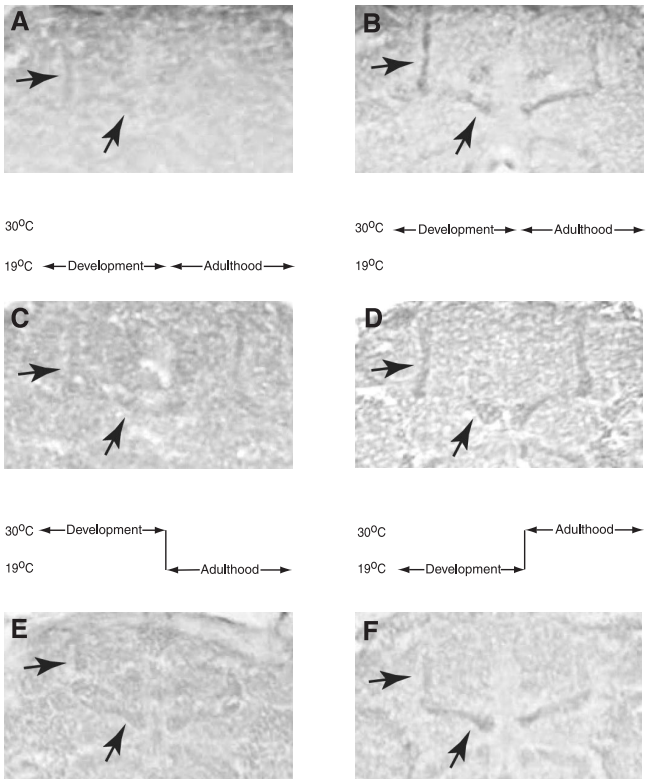

Fig. 3. Temporal control of *rutabaga* expression in the mushroom bodies. Serial frontal brain cryosections were examined for Rutabaga expression by immunohistochemistry as previously described (*22*) and presented here as gray-scale images. Arrows represent the lobes of the mushroom body. (**A**) Background levels of Rutabaga staining in the mushroom bodies of control flies of the genotype rut^{2080}; UAS-*rutabaga*. (**B**) Increased levels of Rutabaga expression in the mushroom bodies of flies of the genotype rut^{2080};c772;UAS-*rutabaga*. (**C**) Background levels of Rutabaga expression in flies of the genotype rut^{2080};c772; GAL80ts/UAS-*rutabaga* that were raised and maintained at 19°C until sectioning. (**D**) Increased levels of Rutabaga staining in the mushroom bodies of flies of the genotype rut^{2080};c772;GAL80ts/ UAS-*rutabaga* that were raised and maintained at 30°C until sectioning. (**E**) Background levels of Rutabaga expression in the mushroom bodies of flies raised through development at 30°C and shifted to 19°C for 7 days as adults. (**F**) Increased levels of Rutabaga expression in the mushroom bodies of flies of the genotype rut^{2080};c772;GAL80ts/UAS-*rutabaga* that were raised at 19°C throughout development and shifted to 30°C for 6 days before sectioning. These flies demonstrated significant improvement of memory scores over flies of the genotype rut^{2080};c772;GAL80ts.

References and Notes

1. G. Roman, R. L. Davis, *Bioessays* **23**, 571 (2001).
2. S. Waddell, W. G. Quinn, *Annu. Rev. Neurosci.* **24**, 1283 (2001).
3. S. E. McGuire, P. T. Le, R. L. Davis, *Science* **293**, 1330 (2001).
4. J. Dubnau, L. Grady, T. Kitamoto, T. Tully, *Nature* **411**, 476 (2001).
5. J. S. de Belle, M. Heisenberg, *Science* **263**, 692 (1994).
6. T. Zars, M. Fischer, R. Schulz, M. Heisenberg, *Science* **288**, 672 (2000).
7. A. H. Brand, N. Perrimon, *Development* **118**, 401 (1993).
8. S. Poser, D. R. Storm, *Int. J. Dev. Neurosci.* **19**, 387 (2001).
9. Y. Dudai, G. Corfas, S. Hazvi, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **162**, 101 (1988).
10. M. S. Livingstone, P. P. Sziber, W. G. Quinn, *Cell* **37**, 205 (1984).
11. H. R. Bourne, R. Nicoll, *Cell* **72** (suppl.), 65 (1993).
12. E. Welker *et al.*, *Science* **271**, 1864 (1996).
13. R. M. Abdel-Majid *et al.*, *Nature Genet.* **19**, 289 (1998).
14. A. Balling, G. M. Technau, M. Heisenberg, *J. Neurogenet.* **4**, 65 (1987).
15. R. Hitier, M. Heisenberg, T. Preat, *Neuroreport* **9**, 2717 (1998).
16. M. Barth, M. Heisenberg, *Learn. Mem.* **4**, 219 (1997).
17. Drosophila stocks carrying P{w^{+mC}=tubP-GAL80ts} transposons have been deposited at the Bloomington Drosophila Stock Center.
18. Materials and methods are available as supporting material on *Science* Online.
19. M. Y. Yang, J. D. Armstrong, I. Vilinsky, N. J. Strausfeld, K. Kaiser, *Neuron* **15**, 45 (1995).
20. J. D. Armstrong, J. S. de Belle, Z. Wang, *Learn. Mem.* **5**, 102 (1998).
21. Heat shock was performed at 32°C.
22. P. L. Han, L. R. Levin, R. R. Reed, R. L. Davis, *Neuron* **9**, 619 (1992).

23. We would like to thank T. Lee for providing the pCaSpeR4 vector that contained the tubulin 1α promoter; S. Elledge for kindly providing yeast strains used in the study; L. Zong for technical assistance with embryo injections; C. Wilson for preparation of the brain cryosections; and G. Chamness and L. Luo for critically reading drafts of the manuscript. Supported by NIH grant nos. NS19904 and GM63929, the R. P. Doherty-Welch Chair in Science (R.L.D.), and an National Research Service Award from the National Institute on Aging (S.E.M.).

Supporting Online Material

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