Sweet taste and nutrient value subdivide rewarding dopaminergic neurons in Drosophila

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Sweet Taste and Nutrient Value Subdivide Rewarding Dopaminergic Neurons in *Drosophila*

Highlights
- Sweet taste and nutrient value recruit different reinforcing dopaminergic neurons
- Sweetness and nutrient value separately reinforce short- and long-term memories
- Reinforcement of short-term memory is not dependent on the state of hunger
- Acquisition and retrieval of long-term memory are hunger state dependent

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In Brief
A small number of dopaminergic neurons in the fly brain are crucial for appetitive memory reinforcement. Huetteroth et al. further subdivide these rewarding neurons into those required for sweet taste reinforcement of short-term memory, nutrient-dependent long-term memory, and others that can artificially reinforce food-independent long-term memory.
Sweet Taste and Nutrient Value Subdivide Rewarding Dopaminergic Neurons in *Drosophila*

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Summary

Dopaminergic neurons provide reward learning signals in mammals and insects [1–4]. Recent work in *Drosophila* has demonstrated that water-reinforcing dopaminergic neurons are different to those for nutritious sugars [3]. Here, we tested whether the sweet taste and nutrient properties of sugar reinforcement further subdivide the fly reward system. We found that dopaminergic neurons expressing the OAMB octopamine receptor [6] specifically convey the short-term reinforcing effects of sweet taste [4]. These dopaminergic neurons project to the β2 and γ4 regions of the mushroom body lobes. In contrast, nutrient-dependent long-term memory requires different dopaminergic neurons that project to the γ5b regions, and it can be artificially reinforced by those projecting to the β lobe and adjacent αr region. Surprisingly, whereas artificial implantation and expression of short-term memory occur in satiated flies, formation and expression of artificial long-term memory require flies to be hungry. These studies suggest that short-term and long-term sugar memories have different physiological constraints. They also demonstrate further functional heterogeneity within the rewarding dopaminergic neuron population.

Results and Discussion

Sweet taste and nutrient value of sugars reinforce learning in *Drosophila* [7, 8]. Octopaminergic neurons specifically convey sweet taste signals [4, 9]. Blocking them impaired short-term memory (STM) reinforced by the sweet but non-nutritious arabinose. In contrast, long-term memory (LTM) formed with sweet and nutritious sucrose was unaffected. Reinforcing octopamine activates a subpopulation of dopaminergic neurons via the Ca2+-coupled α2-adrenergic-like octopamine receptor OAMB. However, despite the evident separation of memory phases with octopamine [4, 10], manipulating dopaminergic neurons has so far impacted sweet taste and nutrient-reinforced memory [3, 4]. We therefore investigated whether octopamine dependence separates rewarding dopaminergic neurons.

*Tbh*mutant flies, lacking octopamine, cannot form STM reinforced with 2 M sucrose [11]. However, a persistent memory slowly emerges after training *Tbh*mutant flies with odor and 1 M sucrose [10], suggesting that nutrient-dependent LTM is likely to be formed in parallel and independent of appetitive STM. Since nutrient-dependent memory can guide behavior as quickly as 2 min after training [7], we first determined whether nutrient memory could be observed in wild-type and *Tbh*mutant flies trained with saturated sucrose, ~5.8 M (Figure 1A). Strikingly, this analysis revealed performance at all times in *Tbh*mutant flies that was statistically different to wild-type immediately after training but indistinguishable from wild-type 30 min, 3 hr, and 24 hr after training. These data are consistent with *Tbh*mutant flies only lacking sweet-taste-reinforced STM [4, 10]. Moreover, they demonstrate that nutrient-dependent (octopamine-independent) memory is observable immediately after training with high sucrose concentrations.

Prior knowledge that octopamine activates rewarding dopaminergic neurons through the OAMB receptor [4] led us to identify R48B04-GAL4 in the FlyLight collection [19]. R48B04-GAL4 is driven by a promoter fragment from the oamb gene (although we acknowledge that this reagent is unlikely to label all oamb-expressing neurons, from here on, we will refer to it as *oambP-GAL4*). We verified the relevance of *oambP-GAL4* neurons by knocking down OAMB expression with UAS-oambRNAi [4, 14]. As expected, these flies completely lacked STM when trained with the sweet and non-nutritious sugar arabinose (Figure 1B). The memory defect was more pronounced than when OAMB was knocked down in dopaminergic neurons with 0104-GAL4 [4], suggesting that *oambP-GAL4* may more accurately label octopamine-responsive dopaminergic neurons than 0104-GAL4 (Figures S1A–S1E). Our initial examination of *oambP-GAL4* revealed expression in approximately 55 rewarding dopaminergic neurons (and ~12 tyrosine hydroxylase (TH)-negative neurons) in the protocerebral anterior medial (PAM) cell cluster that innervate the horizontal mushroom body lobes (Figures 1C–1E, S1A, S1C, and S1D; [5]).

We next tested the contribution of *oambP-GAL4* neurons to saturated sucrose-reinforced memory by blocking their output using the dominant temperature-sensitive UAS-shibire*ts* (UAS-shibire*ts*) transgene [15]. Blocking *oambP-GAL4* significantly impaired STM (Figure 1F). However, LTM performance of *oambP-GAL4/UAS-shibire*ts* flies was indistinguishable from controls, demonstrating a specific loss of STM (Figure 1G), consistent with *Tbh*mutant flies trained with sucrose (Figure 1A).

We also tested a reinforcing role of *oambP-GAL4* neurons by pairing their activation, using UAS-dTrpA1, with odor presentation (Figures 1H and 1I). The dTrpA1-encoded transient receptor potential (TRP) channel conducts Ca2+ and depolarizes neurons when temperature exceeds 25°C [16]. This protocol implanted STM that was statistically different from all controls in both starved and fed flies (Figures 1H and S1F). However, implanted memory did not persist. Performance of *oambP-GAL4/UAS-dTrpA1* flies was indistinguishable from controls 24 hr after training (Figure 1I). Taken together, these
Figure 1. Sweet Taste Reinforces Short-Term Memory via Octopamine Signaling in oambP-GAL4 Dopaminergic Neurons

(A) Tbhm18 flies exhibit defective STM following training with concentrated sucrose (compared to wild-type, p < 0.0001, t test). Residual memory of Tbhm18 flies persists and is statistically indistinguishable from memory in wild-type flies 30 min, 3 hr, and 24 hr after training (all p > 0.7, t test). All n ≥ 12.

(B) Hungry oambP-GAL4;UAS-oambRNAi flies lack STM following training with arabinose (versus controls, p < 0.0001, ANOVA, n ≥ 8).

(C) R48B04 (referred to as oambP-GAL4) labels about 55 dopaminergic neurons that zonally innervate γ1, γ2, γ4, and γ5 of the γ lobe and β02a, β02m, and β02p of the β lobe. 1.5-μm frontal confocal sections at the level of the γ lobe and β lobes are shown; scale bars represent 20 μm. See Figure S1 A for full brain expression.

(D) Schematic of the mushroom body lobes and additional zonal suborganization of the horizontal (red), β (green), and γ (magenta) lobes. The β1, β2 and β1', β2' border their respective α1 and α'1 subregions on the base of the vertical lobes. The exclusively horizontal γ lobe can be split into γ1–γ5 [12].

(E) Illustration of the lobe subregions, highlighting those innervated by oambP-GAL4 dopaminergic neurons (gray).

(legend continued on next page)
Figure 2. Short-Term Memory Reinforcement by Sweet Taste Requires β'2 and γ4 Dopaminergic Neurons

(A–C) Positive intersection between 0104-GAL4 and oambP-lexA with lexAop-FLP,tub>GAL80-stop and UAS-mCD8:GFP labels about 20 neurons. (A) Projection of 20 1-μm confocal sections at the level of γ lobes reveals processes in β', γ1, and γ4. (B) Projection of ten 1-μm confocal sections at the level of β and β' lobes shows innervation in β2m and β2mp and some posterior γ1 from the same cell type as in (A). (C) Projection of ten 1-μm confocal sections of the β' lobe reveals shared 0104-GAL4 and oambP-LexA innervation of β'2m. Scale bars of (A)–(C) represent 20 μm.

(D) Illustration summarizing the zones of the mushroom body innervated by neurons common to 0104-GAL4 and oambP-lexA. (E) oambP neurons not labeled by 0104 are required for STM formation with arabinose. Performance of oambP-LexA/lexAop-shi44 flies is significantly different to controls (p < 0.0001, ANOVA, n = 8). See permissive temperature control in Figure S1G. Illustration demonstrates dopaminergic neurons unique to oambP-lexA/oambP-GAL4. (F and G) Removing oambP neurons from 0104 (oambP-GAL80/20xUAS-6xGFP;0104-GAL4) leaves expression in about 15 neurons innervating γs. (F) The β'2m (red arrow) is otherwise labeled by 0104-GAL4 (Figure S1B), Scale bars represent 20 μm.

(H) Blocking output from the β'2m and γ4 dopaminergic neurons, shown in illustration, during arabinose-reinforced training impairs STM. Performance of oambP-GAL80/0104-GAL4/UAS-shi44 flies was significantly different to controls (p < 0.0001, ANOVA, n = 8). See permissive temperature control in Figure S1H.

(I) Summary of STM formation. Sweet taste engages octopaminergic neurons. Octopamine acts through the OAMB receptor to activate dopaminergic neurons, shown in illustration, during arabinose-reinforced training impairs STM. Performance of oambP-GAL80/0104-GAL4/UAS-shi44 flies was significantly different to controls (p < 0.0001, ANOVA, n = 8). See permissive temperature control in Figure S1H.

(J) Blocking output from the β'2m and γ4 dopaminergic neurons, shown in illustration, during sucrose-reinforced training impairs 24-hr LTM. Performance of 0104-GAL4/UAS-shi44 and oambP-GAL80/0104-GAL4/UAS-shi44 flies was significantly different to controls (p < 0.0002, ANOVA, n = 5–12). See permissive temperature control in Figure S2B.
Both oambP-GAL4 and 0104-GAL4 contain \( \gamma_5 \)-innervating neurons (Figures 1C and S1A–S1E), but the positive intersection does not label them, suggesting that each GAL4 includes unique \( \gamma_5 \) neurons: \( \gamma_{5\text{snarrow}} \) in oambP-GAL4 (Figures 1C, S1A, S1C, and S1D) and \( \gamma_{5\text{sbroad}} \) in 0104-GAL4 (Figures S1B and S1E).

To assess the role of subsets of oambP-labeled neurons, we removed expression in 0104 neurons by combining 0104-GAL4 with UAS-lexA\(_{\text{RNAi}}\), oambP-LexA flies and a lex-Aop-\( \text{shi}^{\text{sa}1} \) transgene, thereby restricting expression to dopaminergic neurons innervating \( \beta_{2\text{m}} \), \( \gamma_4 \), and \( \gamma_5 \) [5]. These flies exhibited no STM following training with sweet-only arabinose at restrictive 33°C (Figure 2E). No significant defect was evident at permissive 23°C (Figure S1G). We also constructed 0104-GAL4/oambP-GAL80 flies in which GAL80 inhibits GAL4-driven gene expression [17] resulting in expression being restricted to \( \beta_{2\text{m}} \) and \( \gamma_{5\text{b}} \) 0104 neurons (Figures 2F–2H). These flies also displayed defective STM following conditioning at restrictive 33°C with arabinose (Figure 2H), while no significant defect was evident at permissive 23°C (Figure S1H). Since blocking \( \gamma_{5\text{an}} \) and \( \gamma_{5\text{bn}} \) neurons with R15A04-GAL4 does not impair STM (Figure S2A), we conclude that sweet taste reinforcement is conveyed by octopaminergic signaling through the OAMB receptor in dopaminergic neurons that innervate the \( \beta_{2\text{an}} \) and \( \gamma_4 \) zones of the mushroom body (Figure 2B).

0104-GAL4 also includes neurons required for nutrient-dependent LTM, which are not in oambP-GAL4. Indeed, blocking 0104-GAL4:oambP-GAL80 neurons with UAS-\( \text{shii}^{\text{sa}1} \) revealed a significant LTM defect (Figure 2J). No defects were apparent at the permissive temperature (Figure S2B). These data indicate that dopaminergic neurons in \( \beta_{2\text{an}} \) and/or \( \gamma_{5\text{bn}} \) are required for nutrient-dependent LTM formation.

We next visually screened for GAL4 lines with expression in PAM dopaminergic neurons that innervate the horizontal mushroom body lobes. We used these and three established PAM lines [3, 4, 18] to express UAS-\( \text{shii}^{\text{sa}1} \) and tested LTM following sucrose-reinforced learning. Blocking 0273, R58E02, or R15A04 neurons during training significantly impaired LTM performance compared to the relevant controls (Figure 3A). In contrast, blocking 0279, 0804, R87D06, or R56H09 neurons did not. No significant defects were apparent when R15A04;UAS-\( \text{shii}^{\text{sa}1} \), R58E02;UAS-\( \text{shii}^{\text{sa}1} \), or 0273;UAS-\( \text{shii}^{\text{sa}1} \) flies were trained and tested at permissive 23°C (Figure S2C).

0273 and R58E02 label ~130 and ~90 dopaminergic neurons, respectively, that broadly innervate the horizontal lobes (Figures 3B and 3C; [3, 4]). R15A04 expresses in ~26 dopaminergic neurons projecting to \( \gamma_{5\text{b}} \), \( \beta_1 \), \( \beta_2 \), \( \gamma_{5\text{bn}} \), and \( \gamma_{5\text{an}} \) (Figures 3D, S2D, and S3K). These overlap with 0104 in \( \beta_2 \) and \( \gamma_{5\text{bn}} \). Ineffective GAL4 lines further refine necessary nutrient-reinforcing neurons (Figures S3 and S4A–S4C; Table S1). Briefly, 0279-GAL4 dopaminergic neurons innervate \( \beta_1 \) and \( \beta_2 \) (Figure 3E; [18]). 0804-GAL4 innervates \( \beta_1 \) and \( \gamma_{5\text{bn}} \) (Figures 3F, S2E, and S3L), R87D06-GAL4 project to \( \gamma_4 \) and \( \beta_1 \) (Figures 3G, S2F, and S3M), and R56H09-GAL4 innervates \( \beta_{2\text{m}} \) and \( \gamma_{5\text{bn}} \) (Figures 3H, S2G, and S3J). These negative data indicate that \( \beta_2 \) and \( \gamma_{5\text{bn}} \) innervation is dispensable (Figures 3A, 3E–3H, and S4C). Therefore, we conclude that nutrient reinforcement requires dopaminergic neurons innervating \( \gamma_{5\text{bn}} \) (Figure 3I).

Artificially activating large groups of 0273 or R58E02 dopaminergic neurons paired with odor formed robust appetitive memory [3, 4]. We therefore tested for subsets that were sufficient to reinforce LTM (Figure 3J). We combined each GAL4 with UAS-dTrpA1 and paired dTrpA1-activating 33°C with odor. Surprisingly, 0273, R58E02, 0104, 0279, R15A04, 0804, and R87D06 produced LTM performance that was statistically different to their relevant control flies, whereas R56H09 did not (Figure 3J). Notably, 0279, 0804, and R87D06 neurons, which were not required for sucrose LTM, reinforced artificial 24-hr memory. These and all other LTM-competent lines (0273, R58E02, 0104, R15A04) include dopaminergic innervation of the \( \beta \) lobe or adjacent \( \gamma_1 \) lobe (Figures 3B–3G, S1B, S1E, and S4C; Table S1), whereas those that cannot implant LTM lack projections to these regions (R4BD04, R56H09; Figures 1C–1E, 3H, S1A, S1C, S1D, S3J, and S4C; Table S1). Therefore, we conclude that artificial LTM can be formed by dopaminergic neurons innervating \( \gamma_1 \), \( \beta_1 \), and \( \beta_2 \) (Figure 3K).

Furthermore, removal of STM-reinforcing oambP-LexA neurons (Figures 1H and 1I) from 0804 (Figure S4A) leaves expression in only two neurons innervating \( \beta_2 \) (Figure S4B), suggesting that these alone may provide sufficient reinforcement for appetitive LTM. Such localization of lasting reinforcement is consistent with the importance of \( \beta_1 \) neurons for LTM and its retrieval [20–23].

Given the discordance between the \( \gamma_{5\text{bn}} \) neurons required for sucrose LTM and those targeting \( \gamma_1 \), \( \beta_1 \), and \( \beta_2 \) that can reinforce persistent memory, we tested the food relevance of implanted memories. Expression of sugar-reinforced memory, but not water-reinforced memory, can be suppressed by feeding flies after training [5]. Feeding suppressed LTM performance of 0104;UAS-dTrpA1, R15A04; UAS-dTrpA1, 0804;UAS-dTrpA1, and R87D06;UAS-dTrpA1 flies to levels that were statistically indistinguishable from their respective-fed controls (Figure 4A). In contrast, significant performance remained in 0279;UAS-dTrpA1 and 0273;UAS-dTrpA1 flies. Therefore, memory reinforced in the \( \beta \) and adjacent \( \gamma_1 \) regions by 0104, R15A04, 0804, and R87D06 neural activation mimics sucrose-reinforced memory, whereas 0279-implanted memory has different properties.

Although flies ordinarily need to be hungry to form sugar-reinforced appetitive memory, prior experiments and those here demonstrate that appetitive STM can be formed in fed flies by pairing octopaminergic or dopaminergic neuron activation with odor presentation (Figure S1F; [3, 4]). We therefore tested whether nutrient-dependent LTM could also be formed artificially in food-satiated flies. We analyzed food-relevant 0104-formed and R87D06-formed memory and non-food satiable 0279-formed and 0273-formed memory in parallel. Strikingly, 0104, R87D06, and 0279 activation did not form LTM in satiated flies, whereas 0273;UAS-dTrpA1 flies exhibited robust LTM (Figure 4B), which was even evident following 7 days of ad libitum feeding after training (Figure 4C). Satiety therefore also constrains the artificial formation of appetitive LTM. We speculate that some 0273 dopaminergic neurons represent rewarding events other than food.

Taken with prior studies [4, 7, 10], results here demonstrate that the sweet taste and nutrient properties of sugars are independently processed and reinforce memories of different duration. Sweet taste is transduced through octopaminergic neurons whose released octopamine, via the OAMB receptor, activates dopaminergic neurons that project to the \( \beta_{2\text{an}} \) and \( \gamma_4 \) regions of the mushroom body. Octopaminergic reinforcement also modulates the state dependence of STM via the
Figure 3. Dopaminergic Neurons Required for Nutrient-Dependent LTM Differ from Those that Can Artificially Implant LTM

(A) Blocking output from 0273, R58E02, and R15A04 neurons during sucrose training significantly disrupted LTM in starved flies (all p < 0.001, ANOVA, n ≥ 9). See permissive temperature control in Figure S2C. LTM was not statistically impaired by blocking 0279, 0804, R87D06, or R56H09 neurons (p > 0.7, ANOVA, n ≥ 8).

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OCTβ2R receptor that is required in the dopaminergic MB-MP1 neurons [4].

Nutrient-dependent LTM does not involve octopamine [4, 10] or sweet-taste-reinforcing dopaminergic neurons. Nutrient reinforcement instead requires dopaminergic neurons innervating γ5b of the mushroom body, whereas those going to β1, β2, and the adjacent α1 region are sufficient. More work will be required to understand this distributed process, which apparently has an immediate and delayed dynamic (Figure S4D; [7, 10]).

Whereas formation and expression of sweet-taste-reinforced STM is insensitive to satiety state, artificial formation and expression of nutrient-relevant memory require flies to be hungry. Even direct stimulation of the relevant rewarding dopaminergic neurons cannot implant appetitive LTM in food-satiated flies. These experiments suggest that hunger establishes an internal state that permits the nutrient-reinforcing signals to be effective. It will be interesting to understand what the permissive state involves and where it is required. Others have previously described a role for CRTC in enabling hunger-dependent LTM in the fly [24] and promoting persistent memory in the mouse [25]. It therefore seems plausible that such a mechanism might be required in the mushroom body neurons to permit nutrient-dependent reinforcement.

Experimental Procedures

Fly Strains

Fly stocks were raised on standard cornmeal food at 25°C and 50%–60% relative humidity. The wild-type Drosophila strain used in this study is Canton-S. The TbHR18 mutant is described [26]. The UAS-mCD8::GFP, the 20xUAS-6xGFP, and the 247-lexA,lexAop-RFP flies are described [17, 19, 27]. The UAS-oambRNAi (strain number 2861GD) was obtained from the Vienna Drosophila Resource Center (VDRC) [14]. The UAS-shito, on the first and third chromosome, and UAS-dTrpA1 transgenic strains are described [15, 16]. The R48B04, R15A04, R87D06, and R56H09 flies [13] were obtained from Bloomington. The R58E02-LexA, R58E02-GAL80, 0104, 0273, and 0279 flies are described [3, 4, 18]. The 0804 fly strain, more correctly named PBac(IT.GAL4)0804, was generated and initially characterized by Marion Sillies and Daryl Gohl as part of the InSITE collection [28].
To visualize native GFP or mRFP, we collected adult flies 2–11 days after eclosion, and brains were dissected in ice-cold 4% paraformaldehyde (PFA). Imaging experiments using UAS-dTrpA1 females were performed with either saturated sucrose or 3 M arabinose as unconditioned stimuli. The odors used were 3-octanol (Sigma) and 4-methylcyclohexanol (Sigma) at 1:1,000 in mineral oil. Artificial memory implantation experiments using UAS-dTrpA1-mediated neural activation were performed as described [1]. Briefly, 8- to 11-day-old flies raised at 20°C were either kept in food vials or starved for 18–22 hr before training. Flies were presented with one odor at the permissive 23°C for 2 min in filter paper-lined tubes and were then transferred into a prewarmed filter paper-lined tube and kept separately overnight with a second odor at dTrpA1 females’ UAS-dTrpA1. Homozygous R48B04 females were crossed to UAS-dTrpA1 females. Heterozygote UAS-dTrpA1/+ controls were generated by crossing UAS-shiR56H09 males to wild-type females and vice versa for both controls in case of 0804.

Behavior Experiments

Appetitive memory was assayed as described [22] with the following modifications. Mixed sex populations of 4- to 8-day-old flies raised at 20°C were tested together in all behavior experiments. Before training, groups of ~100 flies were food deprived for 18–22 hr in a 25-ml vial containing 1% agar and a 20 × 60 mm piece of filter paper. Training was performed with either saturated sucrose or 3 M arabinose as unconditioned stimulus. The odors used were 3-octanol (Sigma) and 4-methylcyclohexanol (Sigma) at 1:1,000 in mineral oil. Artificial memory implantation experiments using UAS-dTrpA1-mediated neural activation were performed as described [1]. Briefly, 8- to 11-day-old flies raised at 20°C were either kept in food vials or starved for 18–22 hr before training. Flies were presented with one odor at the permissive 23°C for 2 min in filter paper-lined tubes and were then transferred into a prewarmed filter paper-lined tube and kept separately overnight with a second odor at dTrpA1 females’ UAS-dTrpA1. Homozygous R48B04 females were crossed to UAS-dTrpA1 females. Heterozygote UAS-dTrpA1/+ controls were generated by crossing UAS-shiR56H09 females to wild-type males. Heterozygote GAL4/+ controls were generated by crossing GAL4 females to wild-type females. We generated flies expressing shiR56H09 in subsets of dopaminergic neurons by crossing UAS-shiR56H09 females to homozygous R48B04, 0104, R48B04-GAL80;0104, R48B04-LexA;0104, 0273, RS8E02, R15A04, 0279, R87D06, or R56H09 males. 0804 resides on the X chromosome; therefore, 0804 females were crossed to UAS-shiR56H09 males. Heterozygote UAS-shiR56H09/+ controls were generated by crossing UAS-shiR56H09 females to wild-type males. Heterozygote GAL4/+ controls were generated by crossing GAL4 males to wild-type females. We generated flies expressing dTrpA1 in R48B04, 0273, RS8E02, 0104, 0279, R15A04, R87D06, or R56H09 neurons by crossing UAS-dTrpA1 females to homozygous R48B04, 0273, RS8E02, 0104, 0279, R15A04, R87D06, or R56H09 males. Homozygous 0804 females were crossed to UAS-dTrpA1 males. Heterozygote UAS-dTrpA1/+ controls were generated by crossing UAS-dTrpA1 females to wild-type males; heterozygote GAL4/+ controls were generated by crossing GAL4 males to wild-type females and vice versa for both controls in case of 0804.

Imaging

To visualize native GFP or mRFP, we collected adult flies 2–11 days after eclosion, and brains were dissected in ice-cold 4% paraformaldehyde solution in PBS (1.86 mM NaH2PO4, 8.41 mM Na2HPO4, and 175 mM NaCl) and fixed for an additional 60 min at room temperature [19]. Samples were then washed 3 × 10 min with PBS containing 0.1% Triton X-100 (PBT) and 2 × 10 min in PBS before mounting in Vectashield (Vector Labs). Imaging of frontal brain views was performed on a Leica TCS SP5 X and a Zeiss LSM 510. The resolution of the image stacks was 1024 × 1024 with 0.5–1.5-μm step size and a frame average of 4. Images were processed in AMIRA 5.3 (Mercury Systems) and Fiji. The immunostaining against TH and GFP was performed as previously described [4].

Supplemental Information

Supplemental Information includes four figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.036.

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