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

**Authors**
Wolf Huetteroth, Emmanuel Perisse, ..., Christopher Burke, Scott Waddell

**Correspondence**
scott.waddell@cncb.ox.ac.uk

**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*

Wolf Huetteroth,1,2 Emmanuel Perisse,1 Suewei Lin,1 Martin Klappenbach,1,3 Christopher Burke,1 and Scott Waddell1,4

1Centre for Neural Circuits and Behaviour, The University of Oxford, Tinsley Building, Mansfield Road, Oxford OX1 3SR, UK
2Zukunftscolleg, University of Konstanz, Universitätstraße 10, 78457 Konstanz, Germany
3Laboratorio de Neurobiología de la Memoria, Departamento de Fisiología y Biología Molecular y Celular, IFIBYNE-CONICET, Pabellón II, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires C1428EGA, Argentina

**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 γ1 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.

*Tbhmt10* mutant flies, lacking octopamine, cannot form STM reinforced with 2 M sucrose [11]. However, a persistent memory slowly emerges after training *Tbhmt10* 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 *Tbhmt10* flies trained with saturated sucrose, ~ 5.8 M (Figure 1A). Strikingly, this analysis revealed performance at all times in *Tbhmt10* 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 *Tbhmt10* 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 [13]. 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 *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*ts1 (UAS-*sh1ts*) transgene [15]. Blocking *oambP-GAL4* significantly impaired STM (Figure 1F). However, LTM performance of *oambP-GAL4;UAS-*sh1ts* flies was indistinguishable from controls, demonstrating a specific loss of STM (Figure 1G), consistent with *Tbhmt10* 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) TbhlM18 flies exhibit defective STM following training with concentrated sucrose (compared to wild-type, p < 0.0001, t test). Residual memory of TbhlM18 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 γ5n 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 S1A for full brain expression.

(D) Schematic of the mushroom body lobes and additional zonal suborganization of the horizontal β (red), β' (green), and γ (magenta) lobes. The β1, β2 and β', 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).
signals. Rewarding dopaminergic neurons contribute nutrient value state-independent sweet taste reinforcement, whereas other specifically convey octopamine-dependent and hunger-neurons and some required for nutrient reinforcement [4].

(F and G) Blocking oambP-GAL4 neuron output with UAS-shaR specifically impairs STM of starved flies trained with sucrose (p < 0.0001, ANOVA, n = 8) (F), whereas it has no effect on 24-hr LTM, as compared to controls (p > 0.1, ANOVA, n = 8) (G). See permissive temperature control in Figure S1B.

(J) Blocking output from the β2m and γ2b subset of 0104-GAL4 neurons during sucrose-reinforced training impairs 24-hr LTM. Performance of 0104-GAL4/UAS-shaR and oambP-GAL80;0104-GAL4/UAS-shaR flies was significantly different to controls (p < 0.0002, ANOVA, n = 5-12). See permissive temperature control in Figure S2B.

Data suggest that oambP-GAL4 dopaminergic neurons specifically convey octopamine-dependent and hunger-state-independent sweet taste reinforcement, whereas other rewarding dopaminergic neurons contribute nutrient value signals.

0104-GAL4 labels octopamine-responsive dopaminergic neurons and some required for nutrient reinforcement [4]. We reasoned that intersecting 0104-GAL4 and oambP-GAL4 would separate sweet and nutrient reinforcement. Common neurons in 0104-GAL4 and oambP-GAL4 can be visualized by combining R48B04-Lexa (i.e., oambP-LexA, which expresses in oambP-GAL4 dopaminergic neurons; Figure S1C) with 0104-GAL4-driven UAS-STOP-GFP (where > represents a FLP-recombinase target sequence) and lexAop-FLP. In these flies, GFP labels 10–20 dopamine neurons innervating the β2am (and γ2b) regions of the mushroom body, in addition to a new class of TH-negative neurons that connect γ1, γ2a, and γ4a in the anterior, median, and posterior lobes (Figure 2A–2D).
Both oambP-GAL4 and 0104-GAL4 contain γ5-innervating neurons (Figures 1C and S1A–S1E), but the positive intersection does not label them, suggesting that each GAL4 includes unique γ5 neurons: γ5 narrow (γ5n) in oambP-GAL4 (Figures 1C, S1A, S1C, and S1D) and γ5 broad (γ5b) 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 RNAi, oambP-LexA flies and a lex-Aop-shst1 transgene, thereby restricting expression to dopaminergic neurons innervating β2m, γ2, and γ5n [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 β2m and γ5b 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 γ5n and γ5b 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 β2m and γ4 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-shst1 revealed a significant LTM defect (Figure 2J). No defects were apparent at the permissive temperature (Figure S2B). These data indicate that dopaminergic neurons in β2m and/or γ5b 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-shst1 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 0273, 0804, R87D06, or R56H09 neurons did not. No significant defects were apparent when R15A04;UAS-shst1, R58E02;UAS-shst1, or 0273;UAS-shst1 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 γ1, β1, β2, γ2m, and γ5n (Figures 3D, S2D, and S3K). These overlap with 0104 in β2m and γ5b. Ineffective GAL4 lines further refine necessary nutrient-reinforcing neurons (Figures S3 and S4A–S4C; Table S1). Briefly, 0279-GAL4 dopaminergic neurons innervate β1 and β2 (Figure 3E; [18]). 0804-GAL4 innervates β2 and γ5n (Figures 3F, S2E, and S3L). R87D06-GAL4 project to γ1 and β1 (Figures 3G, S2F, and S3M), and R56H09-GAL4 innervate β2m and γ5n (Figures 3H, S2G, and S3J). These negative data indicate that β2 and γ5n innervation is dispensable (Figures 3A, 3E–3H, and S4C). Therefore, we conclude that nutrient reinforcement requires dopaminergic neurons innervating γ5b (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 β lobe or adjacent γ1 lobe (Figures 3B–3G, S1B, S1E, and S4C; Table S1), whereas those that cannot implant LTM lack projections to these regions (R48B04, 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 γ1, β1, and β2 (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 β2 (Figure S4B), suggesting that these alone may provide sufficient reinforcement for appetitive LTM. Such localization of lasting reinforcement is consistent with the importance of γ5b neurons for LTM and its retrieval [20–23].

Given the discordance between the γ5b neurons required for sucrose LTM and those targeting β1, β2, and β3 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 β and adjacent γ1 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 β2m and γ5 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 \geq 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 \geq 8).

(legend continued on next page)
OCT2R 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 TbhM18 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-shi, on the first and third chromosome, and UAS-dTrpA1 transgenic strains are described [15, 16]. The R48B04, R15A04, R87D06, and R6H09 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].

(B–H) Mushroom body lobe (red) innervation of 0273-GAL4 (B) [4], R58E02-GAL4 (C) [3], R15A04-GAL4 (D), 0279-GAL4 (E) [18], 0804-GAL4 (F), R87D06-GAL4 (G), and R6H09-GAL4 (H) revealed with UAS-mCD8::GFP. The mushroom body (red) is labeled in each brain with 247-lexA::VP16-driven lexAop-rCD2::mRFP [19]. Scale bars represent 20 μm. Zonal innervation of each line is shown in the corresponding inset illustration.

(l) Summary. Dopaminergic neurons innervating γ5b are essential to reinforce nutrient-dependent LTM.

(J) Pairing odor with dTrpA1 activation of 0273, R58E02, 0104, 0279, R15A04, 0804, and R87D06 dopaminergic neurons forms significant LTM (all p < 0.001, ANOVA, n ≥ 6). No significant memory was formed in R6H09-UAS-dTrpA1 flies (p > 0.3, ANOVA, n ≥ 10). All flies were food deprived before and after training.

(K) Summary. Dopaminergic neurons innervating α1, β1, or β2 (and perhaps β1 and γ5b) are sufficient to form LTM.
eclosion, and brains were dissected in ice-cold 4% paraformaldehyde. To visualize native GFP or mRFP, we collected adult flies 2–11 days after imaging.

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 x 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-octanal (Sigma) and 4-methylcyclohexan-ol (Sigma) at 1:1,000 in mineral oil. Artificial memory implantation experiments using UAS-dTrpA1-mediated neural activation were performed as described [4]. 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 immediately presented with a second odor at dTrpA1-expressing 33°C for 2 min. Flies were then returned to 23°C and tested for immediate memory. To assay 24-hr memory, we transferred trained flies into either food vials or food deprivation vials until testing. For 7-day memory experiments, fed flies were trained and immediately transferred into food vials until memory testing after 7 days. Memory performance was assayed by following the flies 2 min to choose between the odor presented during training. Performance index (PI) was calculated as the number of flies approaching (appetitive memory) the conditioned odor minus the number of flies going to the unconditioned odor divided by the total number of flies in the experiment. A single PI value is the average score from flies of the identical genotype tested with the reciprocal combination of conditioned and unconditioned odor. Statistical analyses were performed using PRISM (GraphPad Software). Overall ANOVA was followed by planned pairwise comparisons between the relevant groups with a Tukey honestly significant difference (HSD) post hoc test. All experiments are n ≥ 8 unless stated otherwise.

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.

Acknowledgments

We thank Y. Huang and R. Brain for technical support and the Bloomington Stock Center, VDRC, T. Claudin, D. Gohl, M. Silles, G. Rubin, and the Janelia Farm project for flies. We thank members of the S.W. and G. Miesenböck laboratories for discussion. W.H. is supported by a ZIF Marie Curie 2-year fellowship. M.K. and S.L. were funded by a Wellcome Trust Senior Research Fellowship in the Basic Biomedical Sciences and by funds from the Gatsby Charitable Foundation and Oxford Martin School.

Received: September 25, 2014
Revised: December 8, 2014
Accepted: January 15, 2015
Published: February 26, 2015

References

1. Schultz, W. (2010). Subjective neuronal coding of reward: temporal value discounting and risk. Eur. J. Neurosci. 31, 2124–2135.
2. Bromberg-Martin, E.S., Matsumoto, M., and Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68, 815–834.
3. Liu, C., Plaçais, P.Y., Yamagata, N., Pfeiffer, B.D., Aso, Y., Friedrich, A.B., Siwanowicz, I., Rubin, G.M., Prent, T., and Tanimoto, H. (2012). A subset of dopamine neurons signals reward for odor memory in Drosophila. Nature 488, 512–516.
4. Burke, C.J., Huetteroth, C., Oswald, D., Perisse, E., Krashes, M.J., Das, G., Gohi, D., Silles, M., Certel, S., and Waddell, S. (2012). Layered reward signalling through octopamine and dopamine in Drosophila. Nature 492, 433–437.
5. Lin, S., Oswald, D., Chandra, V., Talbot, C., Huetteroth, C., and Waddell, S. (2014). Neural correlates of water reward in thirsty Drosophila. Nat. Neurosci. 17, 1536–1542.
6. Han, K.A., Millar, N.S., and Davis, R.L. (1998). A novel octopamine receptor with preferential expression in Drosophila mushroom bodies. J. Neurosci. 18, 3650–3658.
7. Burke, C.J., and Waddell, S. (2011). Remembering nutrient quality of sugar in Drosophila. Curr. Biol. 21, 746–750.
8. Rohwerder, A., Pfitzenmaier, J.E., Ramsperger, N., Apostolopoulou, A.A., Widmann, A., and Thum, A.S. (2012). Nutritional value-dependent and nutritional value-independent effects on Drosophila melangaster larval behavior. Chem. Senses 37, 711–721.
9. Selcho, M., Pauls, D., Huser, A., Stocker, R.F., and Thum, A.S. (2014). Characterization of the octopaminergic and tyraminergic neurons in the central brain of Drosophila larvae. J. Comp. Neurol. 522, 3845–3850.
10. Das, G., Klappeisen, M., Vrontou, E., Perisse, E., Clark, C.M., Burke, C.J., and Waddell, S. (2014). Drosophila learn opposing components of a compound food stimulus. Curr. Biol. 24, 1723–1730.
11. Schwaerzel, M., Monastirioti, M., Scholz, H., Friggø-Grelin, F., Birman, S., and Heisenberg, M. (2003). Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. J. Neurosci. 23, 10495–10502.
12. Tanaka, N.K., Tanimoto, H., and Ito, K. (2008). Neuronal assemblies of the Drosophila mushroom body. J. Comp. Neurol. 508, 711–755.
13. Jenett, A., Rubin, G.M., Ngo, T.T., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B.D., Cavallaro, A., Hall, D., Jeter, J., et al. (2012). A GAL4-driver line resource for Drosophila neurobiology. Cell Rep. 1, 1049–1056.
14. Dietzel, G., Chen, D., Schnorrer, F., Su, K.C., Barninova, Y., Feliner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448, 151–156.
15. Kitamoto, T. (2001). Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. J. Neurobiol. 47, 81–92.
16. Hamada, F.N., Rosenvinge, M., Kang, K., Pulver, S.R., Ghezzi, A., Jegla, T.J., and Garrity, P.A. (2008). An internal thermal sensor controlling temperature preference in Drosophila. Nature 454, 217–220.
17. Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22, 451–461.
18. Perisse, E., Yin, Y., Lin, A.C., Lin, S., Huetteroth, W., and Waddell, S. (2013). Different kenyon cell populations drive learned approach and avoidance in Drosophila. Neuron 79, 945–956.
19. Pitman, J.L., Huetteroth, W., Burke, C.J., Krashes, M.J., Lai, S.L., Lee, T., and Waddell, S. (2011). A pair of inhibitory neurons are required to sustain labile memory in the Drosophila mushroom body. Curr. Biol. 21, 855–861.
20. Isabel, G., Pascual, A., and Preat, T. (2004). Exclusive consolidated memory phases in Drosophila. Science 304, 1024–1027.
21. Yu, D., Akalal, D.B., and Davis, R.L. (2006). Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. Neuron 52, 845–855.
22. Krashes, M.J., and Waddell, S. (2008). Rapid consolidation to a radish and protein synthesis-dependent long-term memory after single-session appetitive olfactory conditioning in Drosophila. J. Neurosci. 28, 3103–3113.
23. Trannoy, S., Redt-Clouet, C., Dura, J.M., and Preat, T. (2011). Parallel processing of appetitive short- and long-term memories in Drosophila. Curr. Biol. 21, 1647–1653.
24. Hirano, Y., Masuda, T., Nagano, S., Matsuno, M., Ueno, K., Miyashita, T., Horiiuchi, J., and Saitoe, M. (2013). Fasting launches CRTC to facilitate long-term memory formation in Drosophila. Science 339, 443–446.
25. Sekeres, M.J., Mercaldo, V., Richards, B., Sargin, D., Mahadevan, V., Woodin, M.A., Frankland, P.W., and Josselyn, S.A. (2012). Increasing CRTC1 function in the dentate gyrus during memory formation or reactivation increases memory strength without compromising memory quality. J. Neurosci. 32, 17857–17868.
26. Monastirioti, M., Linn, C.E.J., Jr., and White, K. (1996). Characterization of Drosophila tyramine beta-hydroxylase gene and isolation of mutant flies lacking octopamine. J. Neurosci. 16, 3900–3911.
27. Shearin, H.K., Macdonald, I.S., Spector, L.P., and Stowers, R.S. (2014). Hexameric GFP and mCherry reporters for the Drosophila GAL4, Q, and LexA transcription systems. Genetics 196, 951–960.
28. Gohl, D.M., Silies, M.A., Gao, X.J., Bhalariao, S., Luong, F.J., Lin, C.C., Potter, C.J., and Clandinin, T.R. (2011). A versatile in vivo system for directed dissection of gene expression patterns. Nat. Methods 8, 231–237.
29. Shang, Y., Griffith, L.C., and Rosbash, M. (2008). Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the Drosophila brain. Proc. Natl. Acad. Sci. USA 105, 19587–19594.
30. Thistle, R., Cameron, P., Ghorayshi, A., Dennison, L., and Scott, K. (2012). Contact chemoreceptors mediate male-male repulsion and male-female attraction during Drosophila courtship. Cell 149, 1140–1151.
31. Sitaraman, D., Zars, M., Laferriere, H., Chen, Y.C., Sable-Smith, A., Kitamoto, T., Rottinghaus, G.E., and Zars, T. (2008). Serotonin is necessary for place memory in Drosophila. Proc. Natl. Acad. Sci. USA 105, 5579–5584.
32. Nicolai, L.J., Ramaekers, A., Raemaekers, T., Drozdzecki, A., Mauss, A.S., Yah, J., Landgraf, M., Annaert, W., and Hassan, B.A. (2010). Genetically encoded dendritic marker sheds light on neuronal connectivity in Drosophila. Proc. Natl. Acad. Sci. USA 107, 20553–20558.
33. Oswald, D., Fouquet, W., Schmidt, M., Wichmann, C., Mertel, S., Depner, H., Christiansen, F., Zube, C., Quentin, C., Körner, J., et al. (2010). A Syd-1 homologue regulates pre- and postsynaptic maturation in Drosophila. J. Cell Biol. 188, 565–579.