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Report

Gamma Neurons Mediate Dopaminergic Input during Aversive Olfactory Memory Formation in *Drosophila*

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Summary

Mushroom body (MB)-dependent olfactory learning in *Drosophila* provides a powerful model to investigate memory mechanisms. MBs integrate olfactory conditioned stimulus (CS) inputs with neuromodulatory reinforcement (unconditioned stimuli, US) [1, 2], which for aversive learning is thought to rely on dopaminergic (DA) signaling [3–6] to DopR, a D1-like dopamine receptor expressed in MBs [7, 8]. A wealth of evidence suggests the conclusion that parallel and independent signaling occurs downstream of DopR within two MB neuron cell types, with each supporting half of memory performance. For instance, expression of the Rutabaga (Rut) adenylyl cyclase in γ neurons is sufficient to restore normal learning to *rut* mutants [9], whereas expression of Neurofibromatosis 1 (NF1) in α/β neurons is sufficient to rescue *NF1* mutants [10, 11]. DopR mutations are the only case where memory performance is fully eliminated [7], consistent with the hypothesis that DopR receives the US inputs for both γ and α/β lobe traces. We demonstrate, however, that DopR expression in γ neurons is sufficient to fully support short- and long-term memory. We argue that DA-mediated CS-US association is formed in γ neurons followed by communication between γ and α/β neurons to drive consolidation.

Results

DopR Expression in MB Kenyon Cells Is Sufficient to Fully Support Both Early and Late Phases of Memory

In order to localize the site of conditioned stimulus-unconditioned stimulus (CS-US) association within mushroom bodies (MBs), we took a complementary approach to previous attempts at mapping the dopaminergic (DA) neurons that convey the US inputs to MBs [3, 4, 6, 12]. We used restricted expression of DopR to map the subset of MB neurons in which the US information is received. This strategy takes advantage of the fact that mutations in *DopR* cause a complete loss of short-term memory (STM) performance [7]. We used an established panel of Gal4 lines (each of which yield expression either in all MB Kenyon cell types or in specific subsets; see Figure S1A available online) to drive expression and tested for rescue of the memory defects of two null alleles of *DopR*: *dumb1*, which is an inversion line *In(3LR)234* whose breakpoints were mapped to 67D and 88A-88B [13], and *dumb2*, which is caused by a piggyBac insertion, PBac{WH}DopR^{f02676}, in the first intron of the *DopR* locus [7]. piggyBac (WH) carries a UAS enhancer for Gal4-driven expression of the flanking adjacent gene [14]. We first confirmed (Figure S1) the finding that expression from this UAS enhancer using the *MB247*Gal4 line is sufficient to fully rescue *dumb2* STM deficit [7, 15]. Because all Gal4 lines that express in MBs also yield some expression in other cell types, we also performed a series of experiments to corroborate the conclusion that postdevelopment MB expression per se is sufficient to rescue the STM defect of *DopR* mutants (Figures S1A–S1I). We also established that the rescued memory observed with Gal4-driven overexpression in MBs shares a genetic signature of normal memory, which is that it can be dissected into a *rutabaga* (*rut*)-dependent and a *rut*-independent component (Figure S1E). This supports the contention that the DopR-rescued memory shares established features of normal memory.

We next examined the relationship between DopR expression and two different forms of consolidated memory. We used ten repeated training sessions, either massed together with no rest interval (massed training) or spaced out with a 15 min rest between sessions (spaced training). Massed training induces a long-lived anesthesia-resistant memory (ARM) [16, 17]. Spaced training, in contrast, induces a long-term memory (LTM) that requires CREB-dependent gene expression [16–18]. We first tested memory 24 hr after massed and spaced training and found that both *dumb1* and *dumb2* mutants are devoid of all memory performance at these time points (Figure 1). We then asked whether MB-driven DopR expression is sufficient to support these forms of consolidated memory. For these experiments, we used Gal4 lines ok107 and *MB247*, which are expressed in more than one MB cell subtype. With ok107, we were able to fully restore 24 hr memory after either spaced (Figure 1C) or massed (Figure 1D) training. DopR expression driven by *MB247*Gal4 also rescued the memory performance after both types of training (Figures 1C and 1D). These data indicate that, as with STM, DopR expression in MBs is sufficient to support both forms of consolidated memory, LTM and ARM (see below for additional evidence).

Acute Expression of DopR in γ KCs Alone Is Sufficient to Support All STM

MB Kenyon cells (KCs) can be classified into three major cell subtypes, each of which sends axon projections into distinct MB lobes. The axons of γ neurons project horizontally to form γ lobes. The axons of α/β neurons bifurcate to form a vertical α lobe and a horizontal β lobe. The axons of α′/β′ neurons also bifurcate into a vertical and horizontal branch, but with terminals in spatial domains somewhat distinct from α/β neurons [19]. In order to dissect the cell-type requirements for DopR, we used a panel of Gal4 lines that distinguish the three major subdivisions of the MB (Figure S1A). For α′/β′ lobe expression, we used c305a and g0050 (Figure S1A; [20–22]), which label about half (c305a) or all (g0050) α′/β′ neurons without labeling the other KC classes. For γ lobe expression,

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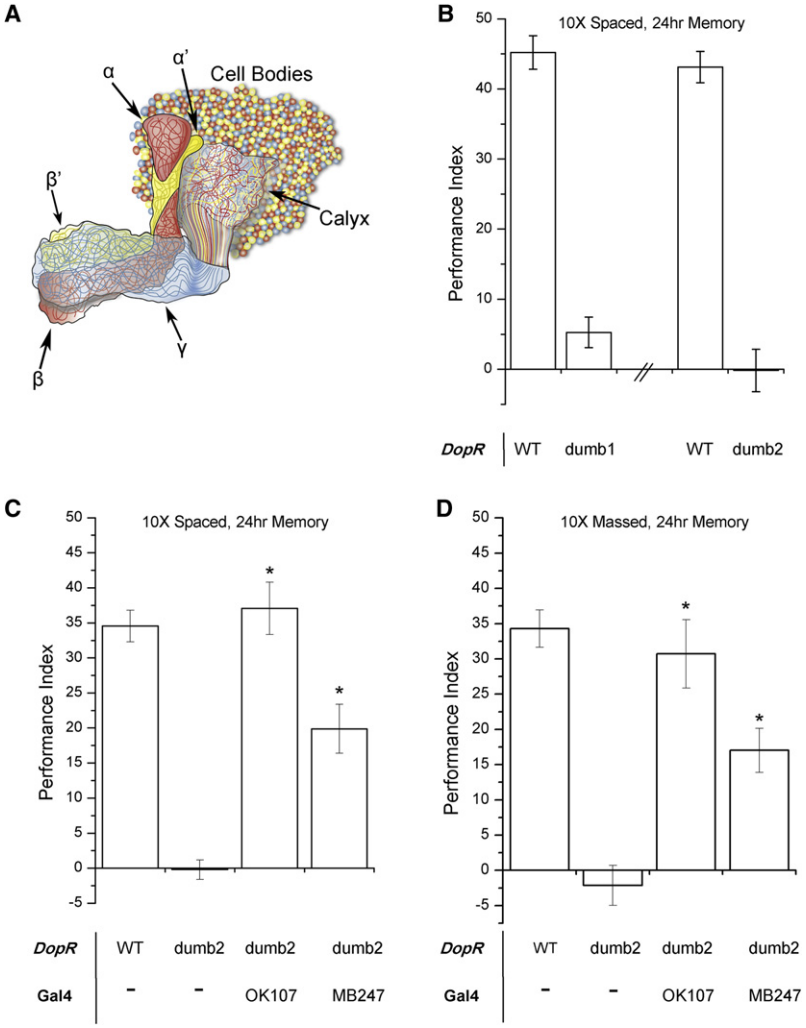

Figure 1. DopR Expression in Mushroom Body Is Sufficient to Support Both Anesthesia-Resistant Memory and Long-Term Memory

(A) An anterior view of the mushroom body in the left hemisphere is illustrated to show the structural features of the three major neuronal cell types. The three major MB Kenyon cell subtypes are color coded red (α/β), yellow (α′/β′), or blue (γ). The Kenyon cells have their cell bodies in the posterior cortex and send projections anteriorly. The dendrites arborize into the calyx. The axons project into five distinct lobes, including two vertical lobes (α and α′) and three medial lobes (β, β′, and γ).

(B) Both *dumb1* and *dumb2* exhibit severely defective LTM (24 hr after 10× spaced training) ($p < 0.05$, $n = 8$ for all groups). At left, the WT control for *dumb1* was Canton-S.

(C) *dumb2* flies with either ok107 or *MB247* MB Gal4 drivers exhibit significantly improved LTM performance (24 hr after 10× spaced training) compared with *dumb2* control flies (*$p < 0.05$, $n = 16$ for all groups).

(D) Expression with the ok107 or *MB247*Gal4 drivers also significantly improved the performance of *dumb2* flies 24 hr after 10× massed training (*$p < 0.05$, $n = 15$ for all groups). The performance with ok107 was not significantly different from that of WT (for spaced training, $p > 0.05$, $n = 16$; for massed training, $p > 0.05$, $n = 15$). Means and standard errors are shown for all groups; wild-type (WT) flies were w^{1118}*(isoCJ1)* unless otherwise noted.

we used NP1131 and 201Y, each of which yields expression in most of the γ neurons, although the expression level with 201Y is relatively low (Figure S1A; [20, 23–25]). NP1131, but not 201Y, also labels a small portion of α′/β′ neurons. 201Y, but not NP1131, labels a small set of core α/β neurons. For α/β lobe expression, we used c739 and NP3061, each of which labels all or most of the α/β lobe without expression in other lobes (Figure S1A; [20, 24, 25]). These cell-type-specific Gal4 drivers generally yield less intense labeling than drivers expressed in multiple MB cell types, such as *MB247*, ok107, c747, and c309 (Figure S1A; [9, 20, 25, 26]). Because the rescue is sensitive to DopR expression level (Figure S1I), we used a *UAS-DopR-cDNA* transgene [27] to supplement the expression derived from the *dumb2* PBac element.

We targeted DopR expression to each MB cell type with lobe-specific Gal4 drivers and tested for STM performance. Remarkably, we found that expression in just γ neurons fully restored STM to *dumb2* mutants. When NP1131 alone was used to drive DopR expression in γ neurons, STM was fully restored to wild-type levels (Figures 2A and 2B) that also were equivalent to performance seen with the combination of NP1131 (γ) and NP3061 (α/β). Similarly, when 201Y alone was used (Figure 2B) to drive expression in γ neurons (and core α/β), we observed rescue of STM performance to levels that were roughly similar to those observed with the combination of 201Y (γ) and c739 (α/β) (compare Figures 2B and S1I). In contrast, we did not observe even partial rescue with NP3061 or c739 (α/β) (Figures 2A and 2C), or with c305a or g0050 (α′/β′) (Figures 2B and 2C). Even the combination of both NP3061 and c739 to yield higher levels of expression in α/β neurons was not sufficient to improve memory of the mutants (data not shown). Although NP1131 and 201Y are relatively specific to γ neurons, both of them have some expression outside of MBs. To show that DopR expression in γ neurons, rather than other cells outside of MBs, rescues the STM performance, we used *MB*Gal80 [22] to suppress the expression of NP1131 within MBs. Flies that carried NP1131, *MB*Gal80, *UAS-DopR-cDNA*, and *dumb2* exhibited performance indices (PIs) that were not significantly different from *dumb2* mutant controls (Figure 2D). Taken together, these findings support the surprising conclusion that DopR expression in γ neurons is necessary and sufficient to fully rescue the STM defect of *dumb2* mutants. In contrast, neither α/β nor α′/β′ expression is necessary or sufficient to significantly restore STM.

DopR Expression in γ KCs Alone Is Sufficient to Support All Phases of Memory

The above findings indicate that memory measured 3 min after one training session can be fully formed with DopR expression restricted to γ neurons. This is consistent with the observation that Rut expression in γ neurons can rescue the STM defect of *rut* mutants but that, unlike *rut*, *DopR* mutants fully disrupt memory performance. The full rescue with γ neuron expression thus suggests that all DA-mediated US signaling for STM is initially mediated by γ neurons. However, in the case

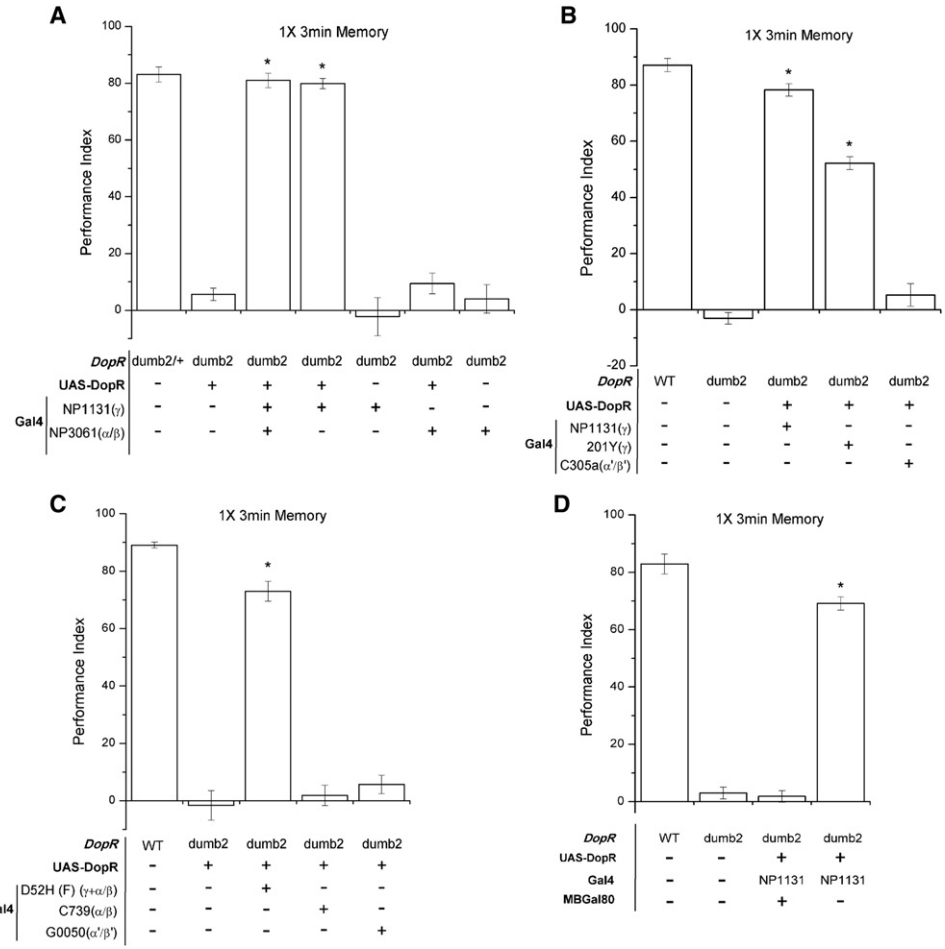

Figure 2. DopR Targeted Expression in γ Kenyon Cells Alone Is Sufficient to Fully Support Short-Term Memory

Memory was tested at 3 min after a single training session. Each γ Gal4 driver, NP1131 (A) or 201Y (B), was sufficient to rescue the STM defect of the *dumb2* mutant in combination with the *UAS-DopR-cDNA* transgene (*p < 0.05, n = 6 for all groups). For NP1131, performance was not significantly different from that of WT (A and B, p > 0.05, n = 6) or the combination of NP1131 and NP3061 (A, p > 0.05, n = 6). In contrast, neither the α/β Gal4 drivers (NP3061, A; c739, C) nor the α′/β′ Gal4 drivers (c305a, B; g0050, C) exhibited significant rescue of STM performance (p > 0.05, n = 6 for all groups). D52H, a Gal4 driver that labels both γ and α/β neurons, provided rescue in combination with the *UAS-DopR-cDNA* transgene (C, *p < 0.05, n = 6). Although NP1131 was sufficient to rescue *dumb2* STM (D, *p < 0.05, n = 6), adding *MB*Gal80 to subtract the MB Kenyon cell expression from NP1131 completely suppressed the NP1131-driven rescue (D, p > 0.05, n = 6). Means and standard errors are shown for all groups; wild-type (WT) flies were w^{1118}*(isoCJ1)* unless otherwise noted.

of Rut, which is thought to act downstream of DopR signaling, memory at later time points after training requires additional expression in α/β lobes [23, 28]. We therefore examined the effects of cell-type-specific DopR expression on memory measured 3 hr after one session of training as well as 24 hr after massed and spaced training.

dumb2 mutant animals did not form detectable 3 hr memory (Figure 3), as was also true for memory measured 24 hr after massed or spaced training (Figures 1, 3, and 4). As with STM measured immediately after one training session, memory measured 3 hr after one training session could be fully rescued with expression in γ neurons using NP1131, and the rescue observed with 201Y, which yields lower levels of γ neuron expression, was nearly as high (Figures 3A and 3B). Here, too, we observed no evidence of rescue when we drove expression with NP3061 (α/β) or c305a (α′/β′). Indeed, the levels of rescue we observed with NP1131 were equivalent to those seen with the combination of NP1131 and NP3061 (Figure 3A). We next conducted similar experiments to probe effects on ARM measured 24 hr after ten cycles of massed training as well as LTM measured 24 hr after spaced training. Here, too, expression with NP1131 (γ) was sufficient to fully rescue these consolidated forms of memory (Figures 3C and 3D). In each case, the levels of performance obtained with only NP1131 were equivalent to those observed in wild-type animals or animals that contained both the NP1131 and NP3061 Gal4 drivers (Figures 3A, 3C, and 3D). And as with 3 min and 3 hr memory, expression of DopR in α/β neurons with NP3061 was not sufficient to provide significant performance improvement (Figures 3C and 3D). Taken together, the above findings support the striking conclusion that DopR expression in only γ KCs is sufficient to support all memory phases, including STM, middle-term memory (MTM), ARM, and LTM. We did not detect any rescue with DopR expression in either α/β alone or α′/β′ alone. Indeed, the levels of rescue observed with γ neurons alone were as high as those observed with γ and α/β combined, and as high as performance of wild-type animals.

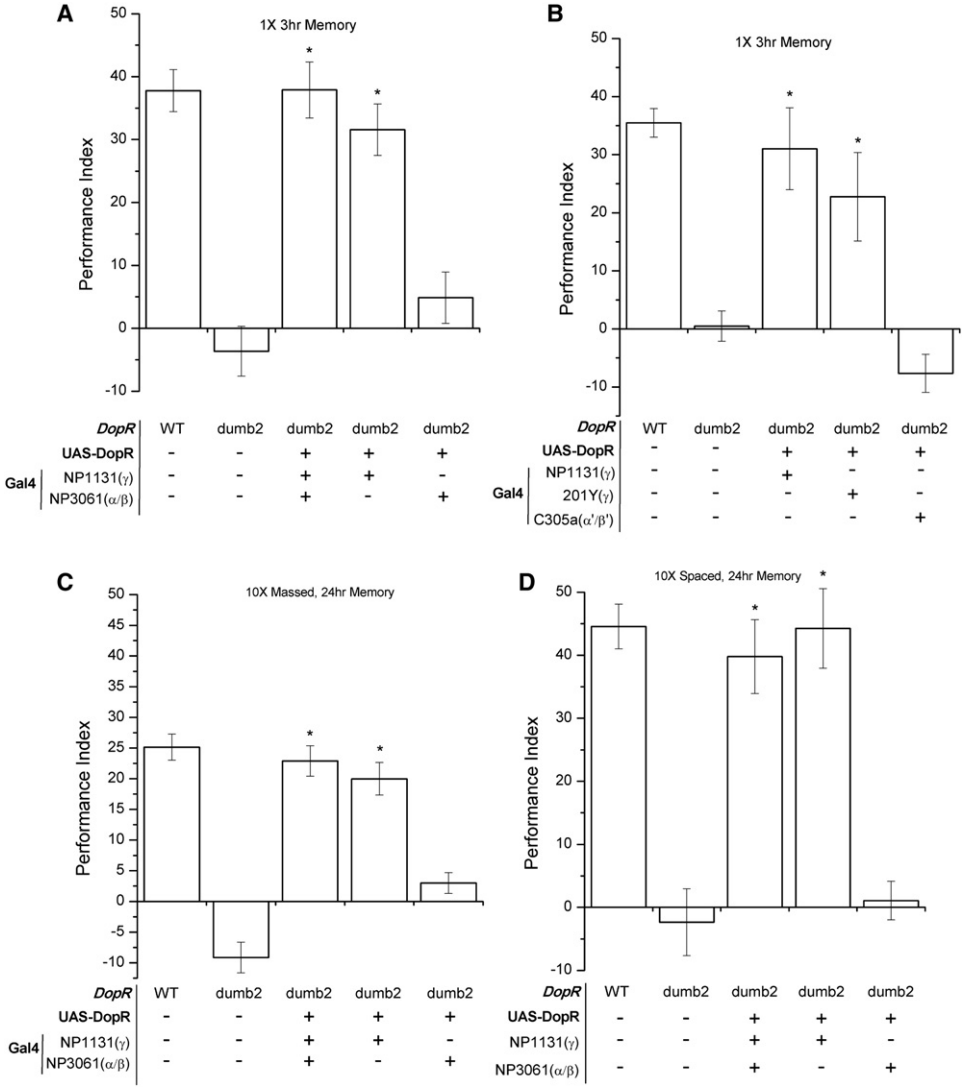

Figure 3. DopR Expression in γ Kenyon Cells Alone Is Sufficient to Support Both Intermediate and Consolidated Memory

Memory was tested either 3 hr after a single training session (A and B) or 24 hr after ten cycles of massed (C) or spaced (D) training. Compared with *dumb2* mutant controls, *dumb2* flies with the NP1131 γ Gal4 driver and *UAS-DopR-cDNA* exhibited full rescue of 3 hr memory (A and B, *$p < 0.05$, n = 6 for all groups), memory 24 hr after massed training (C, *$p < 0.05$, n = 14 for all groups), and memory 24 hr after spaced training (D, *$p < 0.05$, n = 7 for all groups). In each case, the performance observed was equivalent to that of WT (A–D, $p > 0.05$). Similarly, *dumb2* flies with the 201Y γ Gal4 driver and *UAS-DopR-cDNA* also exhibited significant rescue of 3 hr memory (B, *$p < 0.05$, n = 6 for all groups). In contrast, the NP3061 α/β and c305a α′/β′ Gal4 drivers did not show significant rescue compared to *dumb2* controls (A–D, $p > 0.05$, n = 6). Means and standard errors are shown for all groups; wild-type (WT) flies were w^{1118}*(isoCJ1)* unless otherwise noted.

γ Neuron DopR Expression Is a Gateway for US Input to Form Aversive Memory Irrespective of Odor Choice

The Pavlovian olfactory learning paradigm employed here is a discriminative assay in which the animals are trained to associate one odor, the CS+, with electric shock. A second unpaired CS− odor is used as a control. In each experiment, two groups of flies are reciprocally trained to the two odors, and the PI is calculated as an average of the two 1/2 PIs. All experiments in Figures 1–3 utilized 3-octanol (OCT) and 4-methylcyclohexanol (MCH), the two odors that are most commonly used for this assay. Because perception of pure chemical odors is thought to be represented as sparse responses in populations of KCs [29, 30], we wondered whether the sufficiency of γ lobes as a site of DopR input could derive from odor choice if OCT and MCH by chance triggered mostly γ lobes responses. To rule out this sort of explanation, we tested two additional odor combinations among three additional odors that are chemically dissimilar from OCT and MCH. For this series of experiments, we tested the combination of benzaldehyde (BA) paired with OCT and ethyl butyrate (EB) paired with amyl acetate/pentyl acetate (AA). These odors have been successfully used as CSs to induce aversive memory in previous studies [7, 31, 32]. We first tested 3 min STM with EB paired with AA and BA paired with OCT. With these odor combinations, DopR expression in γ neurons with only NP1131 was sufficient to restore STM performance to levels nearly as high as those of wild-type (Figures 4A and 4B). In contrast, expression in α/β or α′/β′ using NP3061 or c305a produced performance levels that were modestly improved (NP3061) or not improved (c305a) relative to

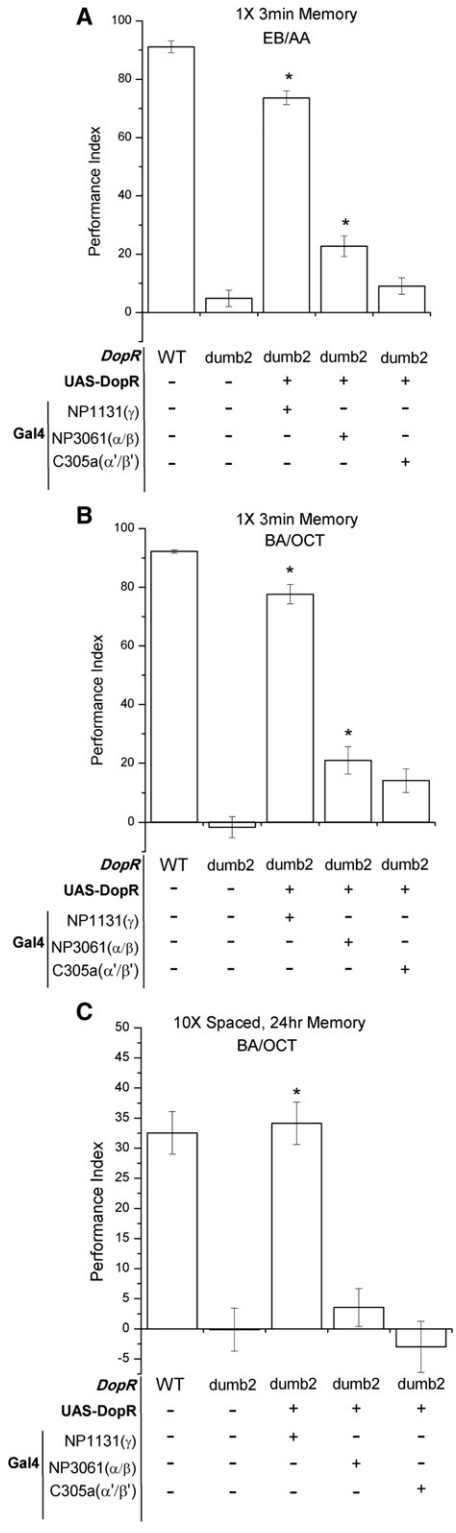

Figure 4. γ Neuron DopR Expression Is Sufficient to Support Aversive Memory Irrespective of Odor Choice

Ethyl butyrate (EB) was paired with amyl acetate (AA) as CS odors for 3 min STM in (A). Benzaldehyde (BA) was paired with octanol (OCT) as CS odors for 3 min STM in (B) and 24 hr LTM (10× spaced training) in (C). In all cases, *dumb2* flies with the NP1131 γ Gal4 driver and *UAS-DopR-cDNA* exhibited rescue of performance compared with *dumb2* controls (A–C, *$p < 0.05$, $n = 8$ for all groups). In contrast, the c305a α′/β′ Gal4 driver did not yield significant rescue in all cases (A–C, $p > 0.05$, $n = 8$ for all groups). NP3061 α/β-driven *dumb2* mutants. Finally, we examined memory 24 hr after spaced training using the combination of BA paired with OCT. Here, too, NP1131-driven γ neuron expression produced normal levels of LTM performance, whereas performance with NP3061 or c305a remained near zero (Figure 4C).

Taken together, these findings demonstrate that DopR signaling for aversive reinforcement of olfactory memory is restricted to the γ neuron subset of MB neurons. This idea is convergent with the established role of MB-MP1 DA neurons, which send fibers into the heel of the MB (which consists largely of γ neurons) and the inner core of the peduncle (which is occupied by α/β neurons) [25, 33]. As an added validation of this model, we used GFP reconstituted across the synapse (GRASP [34, 35]) to visualize connections between these two cell types (Figure S2). When the two halves of GRASP were expressed in MB-MP1 and γ neurons, we observed strong labeling in the heel (Figure S2A) of the MB, consistent with the hypothesis that MB-MP1 forms synapses with γ neurons.

Discussion

Because DopR is thought to mediate the US information [3–7], identification of the spatial requirements of this receptor pinpoints the initial site of CS-US coincidence detection. To date, most genetic and circuit manipulations suggest that olfactory memory performance at a given retention interval can be dissected into distinct and independently disruptable mechanisms acting in parallel in distinct neuronal cell types [1, 36–42]. For example, the STM defects of *rut* and *NF1* can be rescued with expression in γ for *rut* and α/β neurons for *NF1*. Experimental dissections of the circuits required for LTM have suggested a major role for α/β neurons [18, 23, 43, 44] as well as for ellipsoid body (eb) [42] and DAL neurons [45]. Such findings have been interpreted as supporting the idea of independent signaling for parallel memory traces [9, 10, 23] as well as sequential action in different cell types to support a single memory mechanism [22]. Our findings demonstrate that DopR expression in MBs is sufficient to support both *rut*-dependent and -independent forms of CS-US association leading to STM, as well as to consolidated ARM and LTM. This conclusion also generalizes to three different combinations among five different odors, providing strong evidence that the functional distinctions between KC classes are not artifacts caused by differences in the population of neurons involved in coding each odor percept. With each of these odor combinations and memory phases, there also was no case where expression in α/β or α′/β′ populations was sufficient or necessary to provide substantial rescue of *dumb2* mutants.

Together, this set of findings pinpoints the DopR-mediated inputs for STM, MTM, ARM, and LTM to the γ neuron population of MB KCs. This conclusion is consistent with findings from previous attempts to map the subset of DA neurons that convey the US to MBs using either inhibition or activation of neural transmission to block or mimic the US signal [3, 4]. In these studies, the largest magnitude effects were seen with stimulation of MB-MP1, a neuron in the PPL1 cluster of DA neurons (although it should be noted that smaller magnitude

expression yielded minimal levels of STM performance (A and B, *$p < 0.05$, $n = 8$ for all groups), but LTM performance was not rescued (C, $p > 0.05$, $n = 8$). Means and standard errors are shown for all groups; wild-type (WT) flies were *w^{1118}(isoCJ1)* unless otherwise noted.

effects also were seen for several other DA cell types [3]), which is sufficient to substitute for the US. Although inhibition of MB-MP1 neurons has not been demonstrated to block learning [3, 4, 33], these DA neurons likely participate in mediating at least a portion of the US stimulus for aversive conditioning. MB-MP1 neurons project to the base of the peduncle, occupied by the axons of α/β neurons and the heel of the MB, which is comprised largely of γ neurons [25, 33]. As an independent validation of the hypothesis that these MB-MP1 neurons provide direct input to γ neurons, we used the GRASP method [34, 35] to visualize putative synaptic connections in the heel between these two cell types (Figure S2).

The fact that γ lobe expression of DopR is sufficient to restore not only STM but also both ARM and LTM is noteworthy. Previous attempts to map the neural circuits for olfactory memory have revealed roles for α/β lobes in particular for consolidated memory ([18, 23, 28, 40, 43, 44], but cf. [45, 46]). Because massed and spaced training experiments consist of repetitive training rather than the single training trial used for STM and MTM, differences in circuit requirements could in principle derive from training paradigm-dependent differences in the CS-US association circuit, as appears to be true for appetitive reinforcement [28]. But this appears not to be the case for DopR function in aversive reinforcement, because we observed full rescue of these consolidated forms of memory with γ lobe expression of DopR.

How can this conclusion be reconciled with the requirement for downstream signaling molecules within α/β lobe neurons [10, 18, 23], as well as in downstream eb neurons [42] and DAL neurons [45]? We see three possible explanations that are not mutually exclusive. First, it is possible that US information is deconstructed into more than one pathway, mediated by different receptors. These could include additional DA receptors, or other neurotransmitter systems such as serotonin. It is worth noting that DA inputs to MBs also have been implicated in hunger/satiety modulation of appetitive memory retrieval [7, 33], and DopR signaling also has been implicated in several forms of arousal [47] that in principle could represent a component of the reinforcement signal that could be separate from a more specific perceptual representation of the shock experience. Our findings nevertheless lead to the conclusion that any additional US information depends critically on DopR-mediated DA signaling in the γ lobe population of neurons. A second possibility worth considering stems from the finding that output from α/β lobe, eb, and DAL neurons are each required for retrieval depending on the retention interval measured [22, 43, 45, 48]. Thus, we cannot formally rule out a model in which all of the functional impacts of various manipulations of α/β lobe derive from defects in retrieval. This would be difficult to fathom for cases such as NF1 rescue of STM and Rut function for LTM, but in principle this interpretation is possible. The third possibility is that consolidation of the γ lobe CS-US association involves signaling within α/β lobe neurons [10, 18, 23], as well as in downstream eb neurons [42] and DAL neurons [45]. Such a model predicts communication between the γ lobe and the rest of MBs during training and/or afterward (cf. [22, 48, 49]).

Supplemental Information

Supplemental Information includes two figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2012.02.014.

Acknowledgments

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References

1. Keene, A.C., and Waddell, S. (2007). Drosophila olfactory memory: single genes to complex neural circuits. Nat. Rev. Neurosci. *8*, 341–354.
2. Busto, G.U., Cervantes-Sandoval, I., and Davis, R.L. (2010). Olfactory learning in Drosophila. Physiology (Bethesda) *25*, 338–346.
3. Aso, Y., Siwanowicz, I., Bräcker, L., Ito, K., Kitamoto, T., and Tanimoto, H. (2010). Specific dopaminergic neurons for the formation of labile aversive memory. Curr. Biol. *20*, 1445–1451.
4. Claridge-Chang, A., Roorda, R.D., Vrontou, E., Sjulson, L., Li, H., Hirsh, J., and Miesenböck, G. (2009). Writing memories with light-addressable reinforcement circuitry. Cell *139*, 405–415.
5. Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-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.
6. Riemensperger, T., Völler, T., Stock, P., Buchner, E., and Fiala, A. (2005). Punishment prediction by dopaminergic neurons in Drosophila. Curr. Biol. *15*, 1953–1960.
7. Kim, Y.C., Lee, H.G., and Han, K.A. (2007). D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in Drosophila. J. Neurosci. *27*, 7640–7647.
8. Kim, Y.C., Lee, H.G., Seong, C.S., and Han, K.A. (2003). Expression of a D1 dopamine receptor dDA1/DmDOP1 in the central nervous system of Drosophila melanogaster. Gene Expr. Patterns *3*, 237–245.
9. Zars, T., Fischer, M., Schulz, R., and Heisenberg, M. (2000). Localization of a short-term memory in Drosophila. Science *288*, 672–675.
10. Buchanan, M.E., and Davis, R.L. (2010). A distinct set of Drosophila brain neurons required for neurofibromatosis type 1-dependent learning and memory. J. Neurosci. *30*, 10135–10143.
11. Guo, H.F., Tong, J., Hannan, F., Luo, L., and Zhong, Y. (2000). A neurofibromatosis-1-regulated pathway is required for learning in Drosophila. Nature *403*, 895–898.
12. Schroll, C., Riemensperger, T., Bucher, D., Ehmer, J., Völler, T., Erbguth, K., Gerber, B., Hendel, T., Nagel, G., Buchner, E., and Fiala, A. (2006). Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in Drosophila larvae. Curr. Biol. *16*, 1741–1747.
13. Craymer, L. (1984). New mutants report. Drosoph. Inf. Serv. *60*, 234–236.
14. Thibault, S.T., Singer, M.A., Miyazaki, W.Y., Milash, B., Dompe, N.A., Singh, C.M., Buchholz, R., Demsky, M., Fawcett, R., Francis-Lang, H.L., et al. (2004). A complementary transposon tool kit for Drosophila melanogaster using P and piggyBac. Nat. Genet. *36*, 283–287.
15. Lebestky, T., Chang, J.S., Dankert, H., Zelnik, L., Kim, Y.C., Han, K.A., Wolf, F.W., Perona, P., and Anderson, D.J. (2009). Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron *64*, 522–536.
16. Tully, T., Preat, T., Boynton, S.C., and Del Vecchio, M. (1994). Genetic dissection of consolidated memory in Drosophila. Cell *79*, 35–47.
17. Yin, J.C., Wallach, J.S., Del Vecchio, M., Wilder, E.L., Zhou, H., Quinn, W.G., and Tully, T. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell *79*, 49–58.
18. 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.

19. Lee, T., Lee, A., and Luo, L. (1999). Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development *126*, 4065–4076.
20. Aso, Y., Grübel, K., Busch, S., Friedrich, A.B., Siwanowicz, I., and Tanimoto, H. (2009). The mushroom body of adult Drosophila characterized by GAL4 drivers. J. Neurogenet. *23*, 156–172.
21. Lin, H.H., Lai, J.S., Chin, A.L., Chen, Y.C., and Chiang, A.S. (2007). A map of olfactory representation in the Drosophila mushroom body. Cell *128*, 1205–1217.
22. Krashes, M.J., Keene, A.C., Leung, B., Armstrong, J.D., and Waddell, S. (2007). Sequential use of mushroom body neuron subsets during drosophila odor memory processing. Neuron *53*, 103–115.
23. Blum, A.L., Li, W., Cressy, M., and Dubnau, J. (2009). Short- and long-term memory in Drosophila require cAMP signaling in distinct neuron types. Curr. Biol. *19*, 1341–1350.
24. Yang, M.Y., Armstrong, J.D., Vilinsky, I., Strausfeld, N.J., and Kaiser, K. (1995). Subdivision of the Drosophila mushroom bodies by enhancer-trap expression patterns. Neuron *15*, 45–54.
25. Tanaka, N.K., Tanimoto, H., and Ito, K. (2008). Neuronal assemblies of the Drosophila mushroom body. J. Comp. Neurol. *508*, 711–755.
26. Connolly, J.B., Roberts, I.J., Armstrong, J.D., Kaiser, K., Forte, M., Tully, T., and O'Kane, C.J. (1996). Associative learning disrupted by impaired Gs signaling in Drosophila mushroom bodies. Science *274*, 2104–2107.
27. Andretic, R., Kim, Y.C., Jones, F.S., Han, K.A., and Greenspan, R.J. (2008). Drosophila D1 dopamine receptor mediates caffeine-induced arousal. Proc. Natl. Acad. Sci. USA *105*, 20392–20397.
28. 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.
29. Honegger, K.S., Campbell, R.A., and Turner, G.C. (2011). Cellular-resolution population imaging reveals robust sparse coding in the Drosophila mushroom body. J. Neurosci. *31*, 11772–11785.
30. Turner, G.C., Bazhenov, M., and Laurent, G. (2008). Olfactory representations by Drosophila mushroom body neurons. J. Neurophysiol. *99*, 734–746.
31. DasGupta, S., and Waddell, S. (2008). Learned odor discrimination in Drosophila without combinatorial odor maps in the antennal lobe. Curr. Biol. *18*, 1668–1674.
32. de Belle, J.S., and Heisenberg, M. (1994). Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. Science *263*, 692–695.
33. Krashes, M.J., DasGupta, S., Vreede, A., White, B., Armstrong, J.D., and Waddell, S. (2009). A neural circuit mechanism integrating motivational state with memory expression in Drosophila. Cell *139*, 416–427.
34. Gordon, M.D., and Scott, K. (2009). Motor control in a Drosophila taste circuit. Neuron *61*, 373–384.
35. Feinberg, E.H., Vanhoven, M.K., Bendesky, A., Wang, G., Fetter, R.D., Shen, K., and Bargmann, C.I. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. Neuron *57*, 353–363.
36. Blum, A., and Dubnau, J.T. (2010). Parallel processing of olfactory memories in Drosophila. Fly (Austin) *4*, 163–166.
37. Chang, K.T., Shi, Y.J., and Min, K.T. (2003). The Drosophila homolog of Down's syndrome critical region 1 gene regulates learning: implications for mental retardation. Proc. Natl. Acad. Sci. USA *100*, 15794–15799.
38. Pavlopoulos, E., Anezaki, M., and Skoulakis, E.M. (2008). Neuralized is expressed in the alpha/beta lobes of adult Drosophila mushroom bodies and facilitates olfactory long-term memory formation. Proc. Natl. Acad. Sci. USA *105*, 14674–14679.
39. Tan, Y., Yu, D., Pletting, J., and Davis, R.L. (2010). Gilgamesh is required for rutabaga-independent olfactory learning in Drosophila. Neuron *67*, 810–820.
40. Lee, P.T., Lin, H.W., Chang, Y.H., Fu, T.F., Dubnau, J., Hirsh, J., Lee, T., and Chiang, A.S. (2011). Serotonin-mushroom body circuit modulating the formation of anesthesia-resistant memory in Drosophila. Proc. Natl. Acad. Sci. USA *108*, 13794–13799.
41. Tamura, T., Horiuchi, D., Chen, Y.C., Sone, M., Miyashita, T., Saitoe, M., Yoshimura, N., Chiang, A.S., and Okazawa, H. (2010). Drosophila PQBP1 regulates learning acquisition at projection neurons in aversive olfactory conditioning. J. Neurosci. *30*, 14091–14101.
42. Wu, C.L., Xia, S., Fu, T.F., Wang, H., Chen, Y.H., Leong, D., Chiang, A.S., and Tully, T. (2007). Specific requirement of NMDA receptors for long-term memory consolidation in Drosophila ellipsoid body. Nat. Neurosci. *10*, 1578–1586.
43. Isabel, G., Pascual, A., and Preat, T. (2004). Exclusive consolidated memory phases in Drosophila. Science *304*, 1024–1027.
44. Pascual, A., and Préat, T. (2001). Localization of long-term memory within the Drosophila mushroom body. Science *294*, 1115–1117.
45. Chen, C.C., Wu, J.K., Lin, H.W., Pai, T.P., Fu, T.F., Wu, C.L., Tully, T., and Chiang, A.S. (2012). Visualizing long-term memory formation in two neurons of the Drosophila brain. Science *335*, 678–685.
46. Gouzi, J.Y., Moressis, A., Walker, J.A., Apostolopoulou, A.A., Palmer, R.H., Bernards, A., and Skoulakis, E.M. (2011). The receptor tyrosine kinase Alk controls neurofibromin functions in Drosophila growth and learning. PLoS Genet. *7*, e1002281.
47. Van Swinderen, B., and Andretic, R. (2011). Dopamine in Drosophila: setting arousal thresholds in a miniature brain. Proc. Biol. Sci. *278*, 906–913.
48. McGuire, S.E., Le, P.T., and Davis, R.L. (2001). The role of Drosophila mushroom body signaling in olfactory memory. Science *293*, 1330–1333.
49. Dubnau, J., Grady, L., Kitamoto, T., and Tully, T. (2001). Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature *411*, 476–480.