

about the test grating (48 c.p.d.) on the frequency scale. The contrast of the 36 c.p.d. adapting grating was set to half of its threshold contrast, 3.5%. No measurable tilt after-effect was found under this condition (Fig. 2b).

Similarly, in measurements of the elevation in contrast threshold produced by pre-exposure to a range of adapting grating contrasts, at three spatial frequencies spanning the resolution limit, we found that adapting gratings of frequency greater than the resolution limit were more effective than correspondingly subthreshold gratings of lower frequency (although subthreshold gratings in the high but resolvable frequency range were not entirely ineffective). Thus, the cortical requirement for conscious perception seems to depend on spatial frequency, and not only on contrast or signal strength represented at the cortical input.

The idea that limits on visual resolution are partly imposed at the cortical level is supported by evidence that cortically projecting thalamic relay neurons in macaque often respond well to spatial frequencies far above the human resolution limit, in some cases exceeding 100 c.p.d. (ref. 15). If the projection from thalamus to cortex were as precise as the one from retina to thalamus, this would allow the visual system to form a representation of unresolvable patterns at the cortical site of pattern adaptation. The lower spatial-frequency limits for cortical after-effects (70 c.p.d.), as compared with thalamic neurons (100 c.p.d.), may reflect imperfect precision in the projection from thalamus to cortex¹⁶.

In normal vision with incoherent light, diffraction markedly reduces the retinal image contrast for spatial frequencies near the resolution limit. Why should the cortex have orientation-selective mechanisms (or frequency-selective ones¹⁷) that respond to high spatial frequencies that are normally only faintly represented in the retinal image? One answer derives from the view¹⁸ that neural mechanisms might compensate for optical blur: frequency components that have been optically attenuated might even require a reciprocal enhancement of neural sensitivity for their appropriate representation.

But on our evidence, activation of orientation-selective units at the stage of cortical pattern adaptation is not sufficient for perceptual awareness of the pattern orientation. The nature of the added requirement is not clear: one possibility¹⁹ is that information must be relayed from primary visual cortex to another region of the brain to be represented in conscious experience. □

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Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory

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Surgical, pharmacological and genetic lesion studies have revealed distinct anatomical sites involved with different forms of learning. Studies of patients with localized brain damage and work in rodent model systems, for example, have shown that the hippocampal formation participates in acquisition of declarative tasks but is not the site of their long-term storage^{1,2}. Such lesions are usually irreversible, however, which has limited their use for dissecting the temporal processes of acquisition, storage and retrieval of memories^{3,4}. Studies in bees and flies have similarly revealed a distinct anatomical region of the insect brain, the mushroom body, that is involved specifically in olfactory associative learning^{5,6}. We have used a temperature-sensitive *dynamain* transgene, which disrupts synaptic transmission reversibly and on the time-scale of minutes⁷, to investigate the temporal requirements for ongoing neural activity during memory formation. Here we show that synaptic transmission from mushroom body neurons is required during memory retrieval but not during acquisition or storage. We propose that the hebbian processes underlying olfactory associative learning reside in mushroom body dendrites or upstream of the mushroom body and that the resulting alterations in synaptic strength modulate mushroom body output during memory retrieval.

The mushroom body is a central control neuropil that receives multimodal input^{8–10}. In *Drosophila*, one hemisphere of the mushroom body consists of around 2,500 kenyon cells, whose primary afferents convey olfactory input through the antennal-glomerular tract (AGT; Fig. 1). The AGT projects from the antennal lobe, which itself receives olfactory input from the antennae. Mushroom body efferents project to other neuropil regions that are ultimately involved in motor output. Mushroom body neurons are believed to integrate multimodal information, including olfactory stimuli, and to modulate behavioural responses through motor output. Consistent with this anatomical view of the mushroom body, genetic and pharmacological disruptions of mushroom body

neurons interfere with olfactory associative learning but do not perturb sensorimotor responses to the odours or footshock used in the conditioning assay^{5,11}. Normal cyclic AMP signalling in mushroom body neurons is required for olfactory associative learning^{11,12}, indicating that molecular mechanisms in mushroom body neurons may be involved in behavioural plasticity.

Although these findings show that the mushroom body participates in olfactory learning, they do not distinguish among the temporal stages of memory formation (acquisition, storage and retrieval). We have used a mutant transgene of *dynammin* to gain temporal and spatial control of neuronal activity *in vivo*⁷. Mutations in *dynammin* were first discovered in a screen for temperature-sensitive paralytic mutants in *Drosophila*¹³. *shibire* (*shi*) mutants were identified that displayed normal locomotor activity at permissive temperature (20 °C) but became paralysed soon after a shift to restrictive temperature (30 °C). These *shi^{ts}* mutants recovered within minutes after they were shifted back to permissive temperature. Analysis of ultrastructure and function at the neuromuscular junction of *shi^{ts}* mutants has revealed a reversible, temperature-dependent loss of synaptic transmission concomitant with depletion of synaptic vesicles^{14–16}. The *shi* transcription unit encodes the fly homologue of dynamin^{17,18}, a synaptic GTPase required for fission of endocytic vesicles from the plasma membrane. Molecular genetic analysis has indicated that dynamin may assemble into multimeric complexes and that missense mutations in the GTPase domain can affect dynamin function in a dominant-negative fashion^{19,20}. This finding is consistent with the observation that several mutant alleles affecting this protein domain (for example, *shi^{ts1}*) are genetically semidominant.

We expressed a *shi^{ts1}* mutant complementary DNA⁷ in transgenic animals. When overexpressed in a wild-type background, this *UAS–shi^{ts1}* transgene reversibly interferes with neuronal transmission in a temperature-dependent, dominant-negative fashion. We used the Gal4 enhancer-trap system²¹ to manipulate the spatial expression of *UAS–shi^{ts1}*. Analysis of multiple Gal4 drivers has established a panel of lines with preferential expression in mushroom body neurons. These Gal4 driver lines have been used extensively to discern the neural architecture of the mushroom body, direct transgenic expression to the mushroom body for genetic rescue experiments and assess the role of cAMP signalling in the mushroom body during olfactory learning^{11,12,22}. We have built upon this genetic approach to dissociate the spatial and temporal requirements for

synaptic transmission in the mushroom body during acquisition, storage and retrieval of olfactory memory.

Widespread expression of the *UAS–shi^{ts1}* transgene reproduces the reversible temperature-dependent paralysis of *shi^{ts1}* mutants. The MJ85b enhancer line drives Gal4 expression in all neurons of the adult brain except photoreceptors (Fig. 2a)²³. When *UAS–shi^{ts1}* is expressed with this Gal4 driver, transgenic flies rapidly become paralysed after being shifted to restrictive temperature and then recover their locomotion within one minute after a shift back to permissive temperature (Fig. 2c and data not shown)²⁴.

When expression of the *UAS–shi^{ts1}* transgene was limited predominantly to the mushroom body (Fig. 2b), locomotion was normal (Fig. 2d), as were sensorimotor responses to the odours and footshock stimuli used in pavlovian learning assays (Table 1). For these experiments we used two well characterized enhancer-trap lines, C747 and C309. These GAL4 enhancers have been used to drive expression of a dominant-negative form of G α in mushroom body neurons. This disruption abolishes olfactory learning and also has no effect on sensorimotor responses to the odours or footshock¹¹.

We next studied the associative learning produced by temporally pairing odour and footshock in a pavlovian conditioning

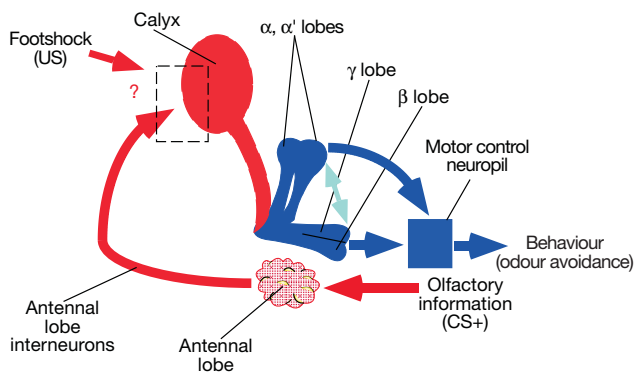


Figure 1 Neural model of olfactory learning in *Drosophila*. The mushroom body is a central control neuropil that receives multimodal input (red) and sends output (blue) to other brain regions involved in the motor control of odour avoidance responses. Feedback within mushroom body neurons (light blue arrow) is likely. Olfactory information is relayed from antennae to mushroom body dendrites (calyx) by antennal lobe interneurons. Concomitant input from footshock through an unknown circuit (?) presumably induces a hebbian process in the mushroom body calyx, thereby modulating synaptic transmission from mushroom body axons (the α -, α' -, β - and γ -lobes) to motor circuits that produce odour avoidance responses. We propose that the hebbian processes underlying olfactory associative learning occur in mushroom body dendrites (box).

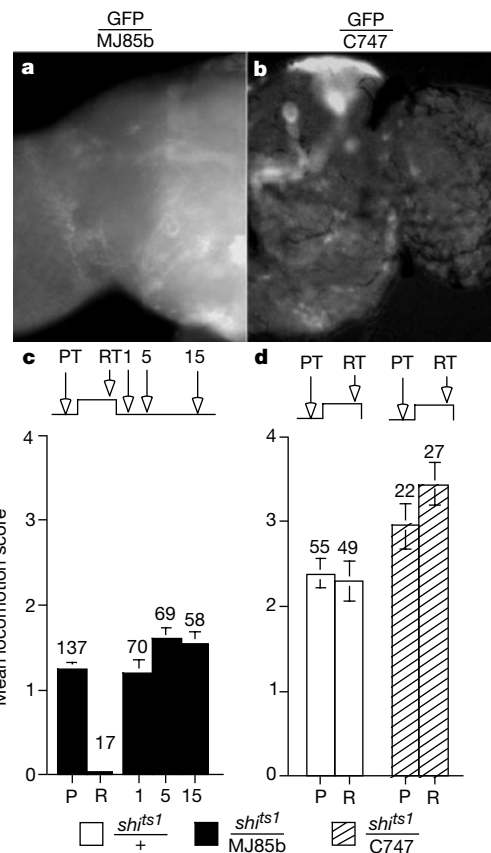


Figure 2 Disruption of neurotransmission produces paralysis when the *UAS–shi^{ts1}* transgene is expressed widely and has no effect when expression is restricted to the mushroom body. **a, b**, Whole mounts of adult brain from transgenic flies carrying a green fluorescent protein (GFP) reporter transgene (see Methods), show **(a)** widespread expression throughout the central brain (left hemisegment) when driven by the MJ85b GAL4 enhancer and **(b)** preferential expression in the mushroom body (right hemisegment) when driven by the C747 GAL4 enhancer. **c**, Locomotor activity in *shi^{ts1}/MJ85b* transgenic flies is abolished 30 min after shifting flies from permissive temperature (20 °C; PT) to restrictive temperature (30 °C; RT; $P < 0.001$). Recovery from paralysis is rapid. Locomotion appears normal again 1 min ($P = 0.166$), 5 min ($P = 0.222$) and 15 min ($P = 0.413$) after shifting transgenic flies back to PT (see Methods). **d**, Locomotion at PT or RT is similar for *shi^{ts1}/+* control flies ($P = 0.546$) and *shi^{ts1}/C747* transgenic flies ($P = 0.182$) after 30 min at RT.

procedure²⁵. Learning in this task was normal at permissive temperature in both *UAS-shi^{ts1}/C747* and *UAS-shi^{ts1}/C309* lines (Fig. 3a, permissive). When these transgenic flies were shifted to restrictive temperature 30 min before training, however, olfactory learning was abolished (Fig. 3a, restrictive). This temperature-dependent disruption of associative learning was not permanent. When *UAS-shi^{ts1}/C747* transgenic flies were shifted to restrictive temperature for 30 min and then back to permissive temperature for 30 min before training and testing, performance was normal (Fig. 3b, recovery).

Proper performance in learning experiments requires a combination of acquisition, storage and retrieval of memory. By limiting the temperature-dependent effects of *UAS-shi^{ts1}* to these three phases of memory formation, we were able to discern whether normal synaptic transmission in mushroom body neurons was required for each aspect of memory processing. We first asked whether synaptic transmission from the mushroom body was required during acquisition. Transgenic *UAS-shi^{ts1}/+*, *UAS-shi^{ts1}/C747* or *UAS-shi^{ts1}/C309* flies were shifted to restrictive temperature for 30 min, trained and then shifted back to permissive temperature immediately afterwards. These flies were then tested at permissive temperature after a 30-min retention period. Compared with control flies that were trained and tested at permissive temperature (Fig. 4a, control), performance was normal in these temperature-shifted flies (Fig. 4a, acquisition). This result indicates that synaptic transmission from mushroom body neurons is not required for acquisition of an odour-footshock association.

We next tested the requirement for mushroom body synaptic transmission during memory storage. Transgenic *UAS-shi^{ts1}/+*, *UAS-shi^{ts1}/C747* or *UAS-shi^{ts1}/C309* flies were trained at permissive temperature, shifted to restrictive temperature immediately after training and then shifted back to permissive temperature for 5 min before testing retention at 30 min. This treatment also did not adversely affect performance (Fig. 4a, storage), indicating that synaptic transmission in mushroom body neurons is not required for storage of early memory.

Finally, we assessed the requirement for mushroom body synaptic transmission during memory retrieval. Transgenic *UAS-shi^{ts1}/+*, *UAS-shi^{ts1}/C747* or *UAS-shi^{ts1}/C309* flies were trained at permissive temperature, shifted immediately afterwards to restrictive temperature and then tested for 30-min retention at the restrictive temperature. In contrast with the previous manipulations, memory retrieval appeared to be defective in these transgenic flies with disrupted synaptic transmission in mushroom body neurons (compare Fig. 4a, control and Fig. 4a, retrieval). This interpretation was complicated, however, by the fact that testing at restrictive temperature reduced 30-min retention scores of the *UAS-shi^{ts1}/+* control group (Fig. 4a). Thus, an alternative explanation for these data was that high temperature nonspecifically reduced performance in all genotypes.

We addressed this question by repeating the above experiment using a 5-min retention interval, for which general effects of restrictive temperature were not apparent (Fig. 3). Transgenic

UAS-shi^{ts1}/+, *UAS-shi^{ts1}/C747* or *UAS-shi^{ts1}/C309* flies were trained at permissive temperature, shifted to restrictive temperature immediately afterwards and then tested for 5-min retention at restrictive temperature. When compared with control flies that were trained and tested at permissive temperature (Fig. 4b, control), the performance of *UAS-shi^{ts1}/+* flies was not affected by testing at restrictive temperature (Fig. 4b, retrieval), as anticipated. In contrast, the performance of *UAS-shi^{ts1}/C747* or *UAS-shi^{ts1}/C309* flies was reduced significantly (Fig. 4b, retrieval). These results are similar to those in Fig. 3a, but were produced even when the transgenic flies were trained at permissive temperature but tested at restrictive temperature. As such, they confirm that disruption of synaptic transmission in the mushroom body blocks memory retrieval. We also used a reversal-retention protocol to dissociate general performance defects common to all the genotypes from a retrieval failure specific to the *UAS-shi^{ts1}/C747* and *UAS-shi^{ts1}/C309* flies. Using this protocol, we observed normal reversal learning at restrictive temperature in the *UAS-shi^{ts1}* control flies, but still observed defective retrieval in both experimental genotypes (see Supplementary Information).

Together, these data refine our understanding of mushroom body function during olfactory memory formation. Earlier studies in bees and flies have established that olfactory associative learning requires mushroom body neurons and cAMP signalling within them^{5,11,12}. The mushroom body is involved in associative mechanisms *per se* because neither pharmacological ablation of mushroom body neurons nor genetic perturbation of cAMP signalling within them affects the 'task-relevant' sensorimotor responses (olfactory

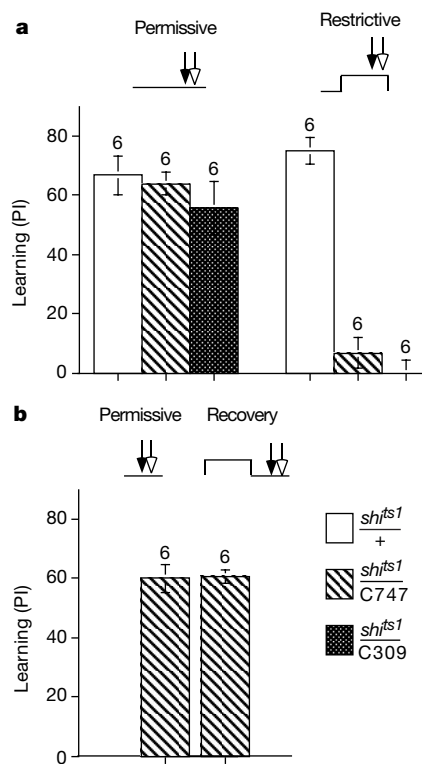


Figure 3 Disruption of neurotransmission in the mushroom body abolishes olfactory associative learning. **a**, When *shi^{ts1}/+*, *shi^{ts1}/C309* and *shi^{ts1}/C747* transgenic flies are trained (filled arrows) in a pavlovian olfactory conditioning assay (see Methods) and then tested (open arrows) immediately thereafter at PT (Permissive; see stimulus schedules above each panel), learning levels are high and similar among genotypes. When transgenic flies are shifted to RT (Restrictive) for 30 min and then trained and tested immediately thereafter, learning levels remain high in *shi^{ts1}/+* flies ($P = 0.355$) but are abolished in *shi^{ts1}/C309* ($P < 0.0001$) and *shi^{ts1}/C747* ($P < 0.0001$) flies. **b**, When *shi^{ts1}/C747* flies are shifted to RT for 30 min and then back to PT for a 30-min recovery before training and testing (Recovery), learning remains high ($P = 0.869$).

Table 1 Sensorimotor responses in *UAS-shi^{ts1}* flies

Genotype (temp.)	SR	OA (MCH)	OA (OCT)
<i>UAS-shi^{ts1}/+</i> (20°C)	67 ± 9	63 ± 7	84 ± 4
<i>UAS-shi^{ts1}/747</i> (20°C)	56 ± 7	54 ± 11	63 ± 6
<i>UAS-shi^{ts1}/309</i> (20°C)	61 ± 13	59 ± 10	80 ± 7
<i>UAS-shi^{ts1}/+</i> (30°C)	60 ± 10	37 ± 10	58 ± 4
<i>UAS-shi^{ts1}/747</i> (30°C)	54 ± 10	28 ± 5	37 ± 7
<i>UAS-shi^{ts1}/309</i> (30°C)	55 ± 9	22 ± 3	44 ± 2

Disruption of synaptic transmission in the mushroom body does not affect the 'task-relevant' sensorimotor responses (olfactory acuity, OA, and shock reactivity, SR) required for proper performance in pavlovian assays. The flies' 'task-relevant' abilities to sense and escape from the odours (olfactory acuity) or footshock (shock reactivity) were quantified in the T-maze (see Methods). Sensorimotor responses to footshock and both odours (OCT and MCH) were quantified at both restrictive and permissive temperatures. No significant differences were detected among genotypes at either temperature, although there was an overall effect of temperature on odour avoidance responses.

acuity and shock reactivity) necessary to perform this task. Connolly *et al.*¹¹ also established the spatial specificity of the enhancer-trap lines used here to drive *UAS-shi^{ts1}* expression in the mushroom body. Unlike these earlier studies, however, our use of the temperature-sensitive *UAS-shi^{ts1}* transgene permitted rapid and reversible disruption of synaptic transmission in otherwise intact mushroom body neurons.

In addition to its role in synaptic vesicle recycling, dynamin also participates in endocytotic protein trafficking of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, a process that occurs on the timescale of several minutes and has been proposed to underlie the acquisition and/or early maintenance of long-term synaptic depression^{26,27}. An analogue of long-term depression may represent a cellular substrate of olfactory learning in *Drosophila*, but our disruption of dynamin function in mushroom body neurons did not affect either acquisition or storage of olfactory memory. Hence, our observations are more consistent with the theory that memory retrieval requires acute synaptic transmission from mushroom body axons, although the possibility that receptor internalization underlies memory retrieval cannot be ruled out formally.

These findings have several implications for our understanding of olfactory memory processing in *Drosophila*. First, they indicate that acquisition and storage of olfactory memory may both occur 'upstream' of synaptic transmission from mushroom body neurons. When combined with observations that cAMP signalling in the mushroom body is required for olfactory learning^{11,12}, our data

indicate that the hebbian processes underlying associative learning may reside within mushroom body neurons. Moreover, they are consistent with the idea that *rutabaga* adenylyl cyclase in mushroom body dendrites (calyces) may be the 'coincidence detector' for associations between the conditioned and unconditioned stimuli²⁸. This notion does not exclude the possibility that other hebbian processes upstream of the mushroom body, such as in the antennal lobes⁶, contribute to this form of learning. Finally, although mushroom body architecture suggests the presence of intrinsic feedback (Fig. 1)¹⁰, our data indicate that acquisition and memory storage during the first 30 min after training do not depend upon ongoing (reverberating) neuronal activity within the mushroom body. A recent study of *amnesiac* mutants is consistent with this view, although a later stage of memory formation (middle-term memory at 60-min retention) appears to depend on neural activity from (dorsal paired medial) neurons outside the mushroom body^{29,30}. In contrast, memory retrieval appears to be a neural process that requires transmission within (for example, feedback) and/or from mushroom body neurons. We propose that learning-driven changes in synaptic weight in mushroom body calyces contribute to a modification of output from the mushroom body during memory retrieval.

Understanding how newly acquired experiences are processed and coded will involve discovery of the molecular, cellular, network and behavioural properties underlying learning and memory formation. Rapidly reversible disruption of synaptic transmission in defined brain subregions permits a synthesis of temporally dynamic processes with identified anatomies. Computational models of cognition will derive from this integrative neurogenetic approach. □

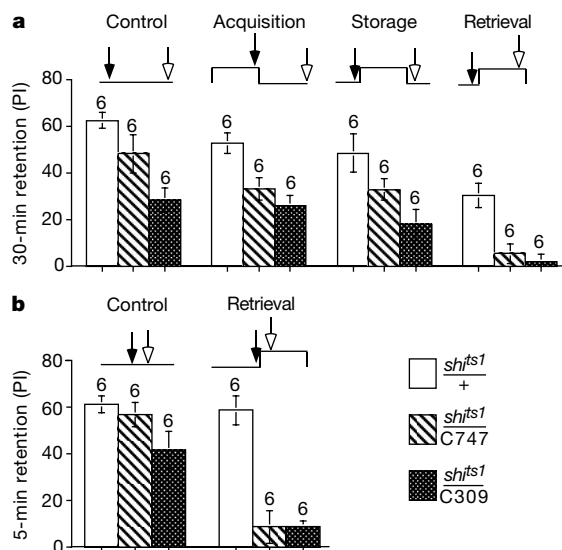


Figure 4 Disruption of neurotransmission in the mushroom body abolishes retrieval but not acquisition or storage of olfactory associative memory. **a**, Control: when *shi^{ts1}/+*, *shi^{ts1}/C309* and *shi^{ts1}/C747* transgenic flies are trained and tested 30 min later at PT, all three groups show learning, although retention levels are lower in *shi^{ts1}/C309* ($P < 0.001$) but not in *shi^{ts1}/C747* flies ($P = 0.067$) than in *shi^{ts1}/+* controls. Acquisition: when flies are shifted to RT for 30 min, trained and then shifted back to PT for 30 min before testing, retention in all three genotypes is similar to that at PT ($P = 0.212$, 0.757 and 0.056 for *shi^{ts1}/+*, *shi^{ts1}/C309* and *shi^{ts1}/C747*, respectively). Storage: when flies are trained at PT, shifted to RT for a 30-min retention interval and then shifted back to PT for 5 min before testing, performance levels are again similar to those at PT ($P = 0.072$, 0.176 and 0.049 for *shi^{ts1}/+*, *shi^{ts1}/C309* and *shi^{ts1}/C747*, respectively). Retrieval: when transgenic flies are trained at PT, shifted to RT immediately and tested for 30-min retention, memory remains in *shi^{ts1}/+* control flies but is near zero in *shi^{ts1}/C309* ($P = 0.607$) and *shi^{ts1}/C747* flies ($P = 0.243$). **b**, Control: when flies are trained and tested 5 min later at PT, all three genotypes display robust performance. Retrieval: when flies are trained at PT, shifted to RT immediately and then tested for 5-min retention, memory scores are unaffected for *shi^{ts1}/+* control flies ($P = 0.77$) but are significantly reduced for *shi^{ts1}/C747* ($P < 0.001$) and *shi^{ts1}/C309* flies ($P < 0.001$).

Methods

Whole-mount expression of GFP

To examine nervous systems of *Drosophila melanogaster* expressing green fluorescent protein (GFP), brains were dissected in 4% paraformaldehyde in PBS. They were fixed by being maintained in this solution for more than 30 min. They were then mounted in Vectashield (Vector Labs) and examined under a Zeiss fluorescent microscope with a far-blue (FITC) filter. *UAS-shi^{ts1}/MJ85b*, *UAS-shi^{ts1}/C747*, *UAS-shi^{ts1}/C309* and *UAS-shi^{ts1}/+* flies were generated by crossing MJ85b/MJ85b²³, C747/C747 and C309/C309 homozygotes or w¹¹¹⁸ (isoCJ1) inbred wild-type flies with *UAS-shi^{ts1}/UAS-shi^{ts1}*; *UAS-shi^{ts1}/UAS-shi^{ts1}* double homozygotes⁷. *UAS-GFP/MJ85b* and *UAS-GFP/C747* flies were generated by crossing *UAS-GFP* homozygotes with MJ85b or C747 homozygotes, respectively. C747 and C309 enhancer trap lines drive preferential expression in the mushroom body, but also yield some expression elsewhere. Hence we have used these two independent lines throughout to focus on mushroom body neurons, the common anatomical site of expression in both lines.

Behavioural analyses

Locomotor activity was measured using countercurrent phototaxis apparatus²⁴ in the absence of light. Under these conditions, tapping flies into the start tube between trials produces an escape response, generally referred to as locomotor reactivity, which decays with time until only spontaneous locomotor activity is expressed. Five runs were done to separate flies, fractionating them into six groups based on the number of times a fly ran into the distal tube. The most active flies ran into distal tubes five times and received a score of five; the least active flies stayed in the original start tube throughout the experiment and received a score of zero. Countercurrent data are binomially distributed. Hence, these data were subjected to an arcsin square-root transformation before subjecting them to analysis of variance. To maintain an experiment-wise error rate of $\alpha = 0.05$ for data in Fig. 2c and d, the adjusted error rate (α') for the six subsequent planned pairwise comparisons was 0.009.

Olfactory associative learning was quantified by subjecting 2–3-day-old adult flies to a pavlovian conditioning procedure²⁵. Groups of about 100 flies received one training session, during which they were exposed sequentially to one odour (conditioned stimulus, CS+) paired with footshock and then a second odour (CS-) without footshock. Conditioned odour avoidance was tested immediately or 5 min or 30 min after training. During the test trial, flies were exposed simultaneously to the CS+ and CS- in a T-maze. After 2 min, flies were trapped in either T-maze arm, anaesthetized and counted. From this distribution, a performance index (PI) was calculated, so that a 50:50 distribution (no memory) yielded a PI of zero and a 0:100 distribution away from the CS+ yielded a PI of 100.

Olfactory acuity was quantified by exposing naive flies to odour (at concentration used for pavlovian training) versus air in the T-maze during a 2-min test trial. Shock reactivity was quantified by placing grids in each arm of the T-maze and then by exposing naive flies to shock versus no shock (at intensity used for pavlovian training) during a 2-min test trial. For both olfactory acuity and shock reactivity, PIs were calculated as above. The

adjusted error rate (α') for the six subsequent planned pairwise comparisons was 0.009. $n = 8$ PIs for each group.

PIs are distributed normally. Hence, these data were analysed by analysis of variance with subsequent pairwise comparisons adjusted to maintain an experiment-wise error rate of $\alpha = 0.05$. In Fig. 3, the error rate for the one subsequent planned comparison was 0.05. In Fig. 4, to maintain an experiment-wise error rate of $\alpha = 0.05$, the adjusted error rates (α') were $P = 0.004$ and 0.01 , respectively, for the 13 subsequent planned pairwise comparisons in Fig. 4a and for the 5 subsequent planned comparisons in Fig. 4b. The number of PIs included in each group mean is listed above the corresponding bar in all figures.

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Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus

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The administration of leptin¹ to leptin-deficient humans, and the analogous *Lep^{ob}/Lep^{ob}* mice, effectively reduces hyperphagia and obesity^{2,3}. But common obesity is associated with elevated leptin, which suggests that obese humans are resistant to this adipocyte hormone. In addition to regulating long-term energy balance, leptin also rapidly affects neuronal activity^{4–6}. Proopiomelanocortin (POMC) and neuropeptide-Y types of neurons in the arcuate nucleus of the hypothalamus⁷ are both principal sites of leptin receptor expression and the source of potent neuropeptide modulators, melanocortins and neuropeptide Y, which exert opposing effects on feeding and metabolism^{8,9}. These neurons are therefore ideal for characterizing leptin action and the mechanism of leptin resistance; however, their diffuse distribution makes them difficult to study. Here we report electrophysiological recordings on POMC neurons, which we identified by targeted expression of green fluorescent protein in transgenic mice. Leptin increases the frequency of action potentials in the anorexigenic POMC neurons by two mechanisms: depolarization through a nonspecific cation channel; and reduced inhibition by local orexigenic neuropeptide-Y/GABA (γ -aminobutyric acid) neurons. Furthermore, we show that melanocortin peptides have an autoinhibitory effect on this circuit. On the basis of our results, we propose an integrated model of leptin action and neuronal architecture in the arcuate nucleus of the hypothalamus.

Previous studies suggest that leptin does not have equal effects on all neuronal subtypes. Acute leptin treatment presumably activates POMC, but not neuropeptide Y (NPY) neurons in the arcuate nucleus of the hypothalamus (ARC), because c-Fos protein is increased only in the former population and *Socs3* messenger RNA is increased in both¹⁰. Furthermore, a population of ARC neurons seems to be inhibited directly by leptin, but the peptide phenotype of these neurons has not been directly established^{11–14}. In addition to leptin receptors, both POMC and NPY neurons express a receptor, MC3-R, for POMC-derived melanocortin peptides¹⁵. The physiological role of this receptor is not well understood, although MC3-R null mice have increased adiposity compared with wild-type mice¹⁶.

To test the hypothesis that leptin selectively activates POMC neurons, we first generated a strain of transgenic mice expressing green fluorescent protein (EGFP; Clontech) under the transcriptional control of mouse *Pomc* genomic sequences, including a region located between –13 kilobases (kb) and –2 kb that is required for accurate neuronal expression¹⁷ (Fig. 1a). Bright green fluorescence (509 nm) was seen in the two central nervous system regions where POMC is produced: the ARC and the nucleus of the solitary tract (data not shown).