Lecture 10 Internal states, memory and choices, circadian rhythms and sleep. 29.11.24

Review TRENDS in Genetics Vol.17 No.12 December 2001 719

What can we teach *Drosophila*? What can they teach us?

Scott Waddell and William G. Quinn

A number of single gene mutations dramatically reduce the ability of fruit flies to learn or to remember. Cloning of the affected genes implicated the adenylyl cyclase second-messenger system as key in learning and memory. The expression patterns of these genes, in combination with other data, indicates that brain structures called mushroom bodies are crucial for olfactory learning. However, the mushroom bodies are not dedicated solely to olfactory processing; they also mediate higher cognitive functions in the fly, such as visual context generalization. Molecular genetic manipulations, coupled with behavioral studies of the fly, will identify rudimentary neural circuits that underly multisensory learning and perhaps also the circuits that mediate more-complex brain functions, such as attention.

flies that are fed the protein-synthesis inhibitor cycloheximide before and after training or in flies that are anesthetized (by cold shock) after training. The remaining half is both unaffected by cycloheximide and is resistant to anesthesia. Apparently, these two components (susceptible to cycloheximide/anesthesia and resistant) comprise all of LTM (Ref. 12). The *rsh* mutation specifically abolishes the anesthesia-resistant component of LTM (ARM) (Refs 9,12), providing our only clue to ARM. *rsh* mutant flies that are fed cycloheximide have no memory at 24 hours[12].

ANNUAL REVIEWS

Annual Review of Neuroscience

The *Drosophila* Mushroom Body: From Architecture to Algorithm in a Learning Circuit

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Keywords

associative learning, pattern separation, dopamine, plasticity, valence, action selection

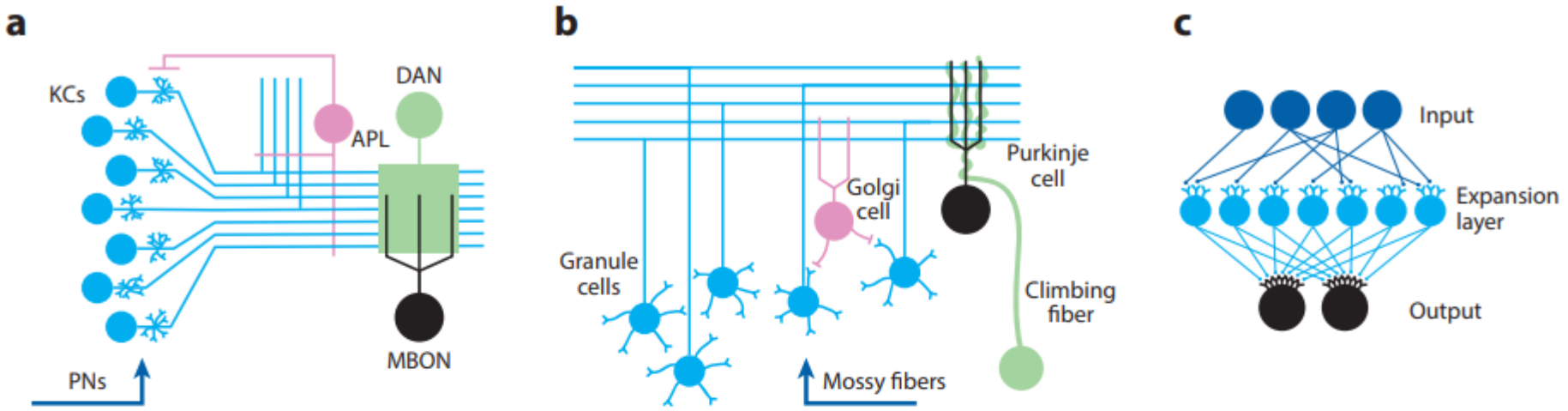

Figure 1

Three-layer networks and pattern separation. (*a*) Mushroom body (MB) circuit. Olfactory input arrives at the dendritic claws of Kenyon cells (KCs) (*light blue*) from projection neurons (PNs) (*dark blue*). KCs project axon bundles to the lobes, where they contact an example mushroom body output neuron (MBON) (*black*). Input from a dopaminergic neuron (DAN) (*green*) drives plasticity at KC>MBON synapses in this compartment. The APL neuron (*pink*) conveys feedback inhibition to KCs. (*b*) Cerebellar circuit. Granule cells (*light blue*) receive input from mossy fibers (*dark blue*) and send parallel fibers to synapse onto Purkinje cells (*black*). Climbing fibers (*green*) wrap around the dendrites of Purkinje cells and carry plasticity-inducing input. Golgi cells (*pink*) provide inhibition to granule cells. (*c*) Perceptron. A small number of input units (*dark blue*) project to a large number of hidden expansion layer units (*light blue*). This expansion layer then converges onto a small number of output units (*black*). (*d*) Channel mixing and thresholding operations. (*Top*) Depiction of tuning curves of multiple input neurons. These are idealized for illustration purposes; actual olfactory tuning properties are more complex. Expansion layer neurons (*light blue*) summate input from different combinations of inputs with different synaptic strengths (*thick line*). Dotted lines represent firing thresholds. (*Bottom*) Illustration of resulting tuning curves. (*e*) Simulation of pattern separation across layers. (*Top*) Responses of 50 input neurons to two stimuli (Stim A and Stim B) chosen to be strongly overlapping. (*Bottom*) Responses of 500 expansion layer neurons created by randomly integrating seven input neurons and thresholding so 5% of expansion layer neurons are active. Response patterns here are nonoverlapping and decorrelated.

- **Purkinje was in Prague. Masaryk University was Purkinje University during the Communist period.**

Figure 1

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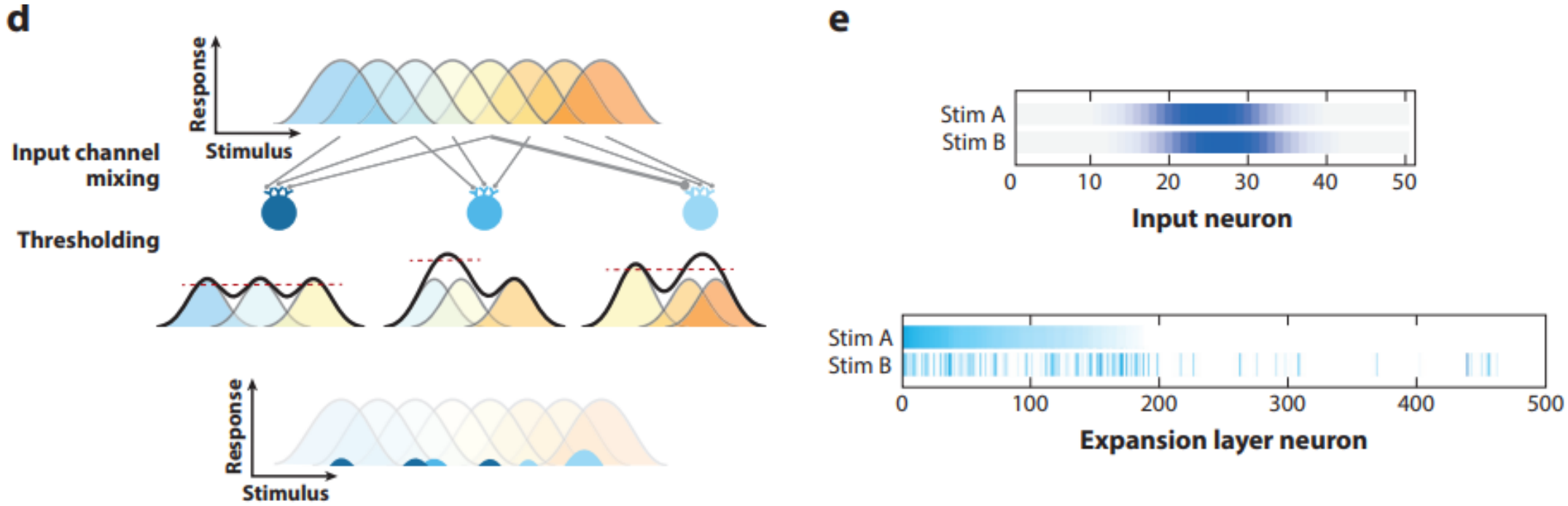


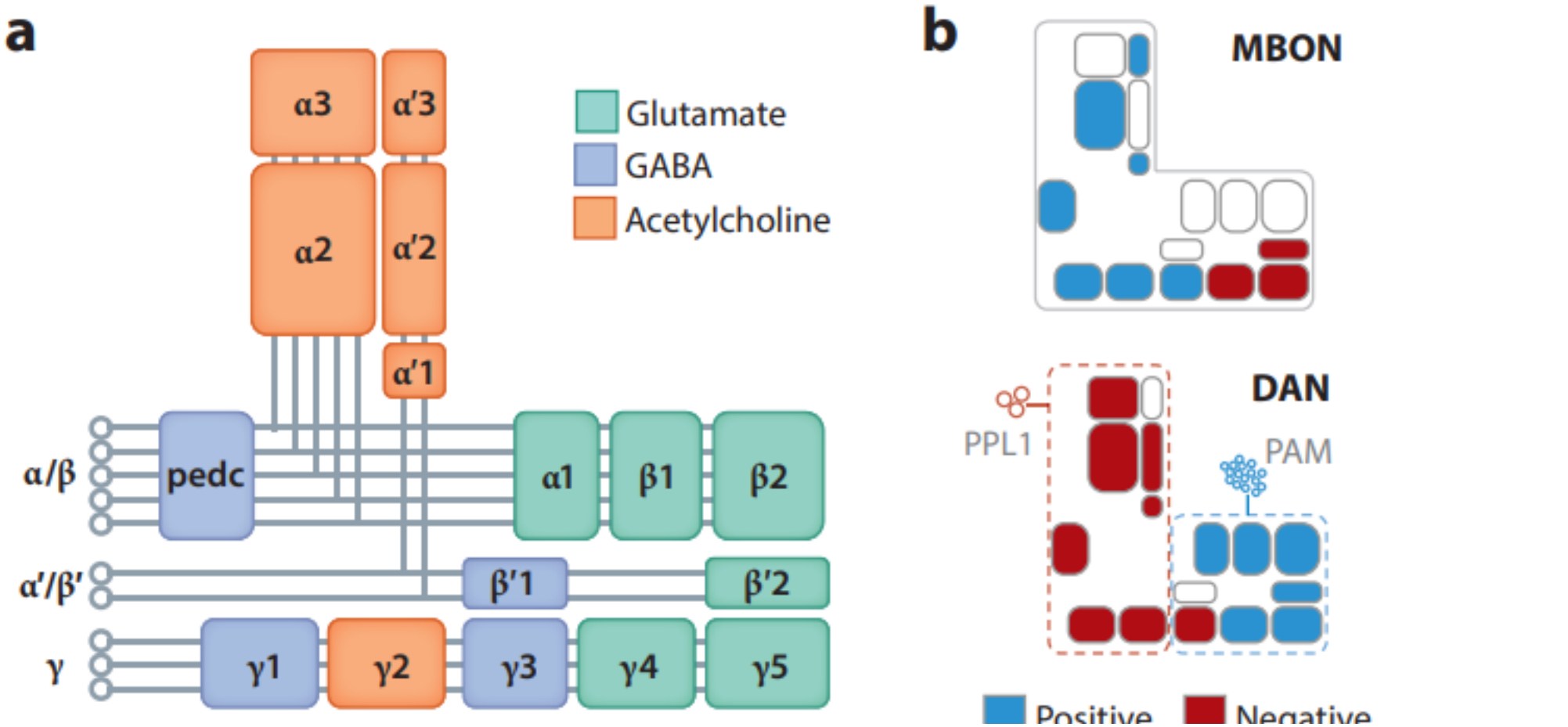
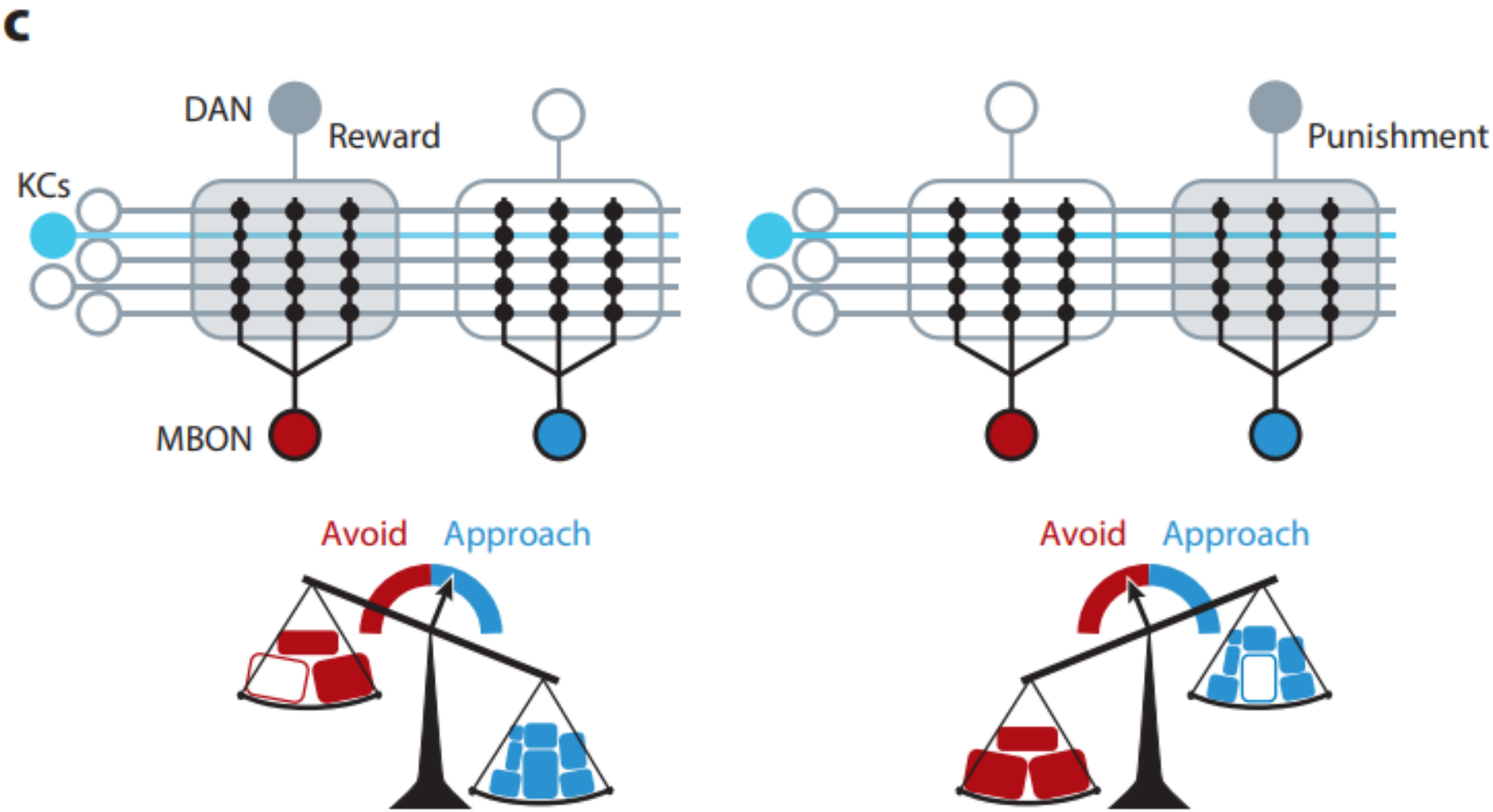


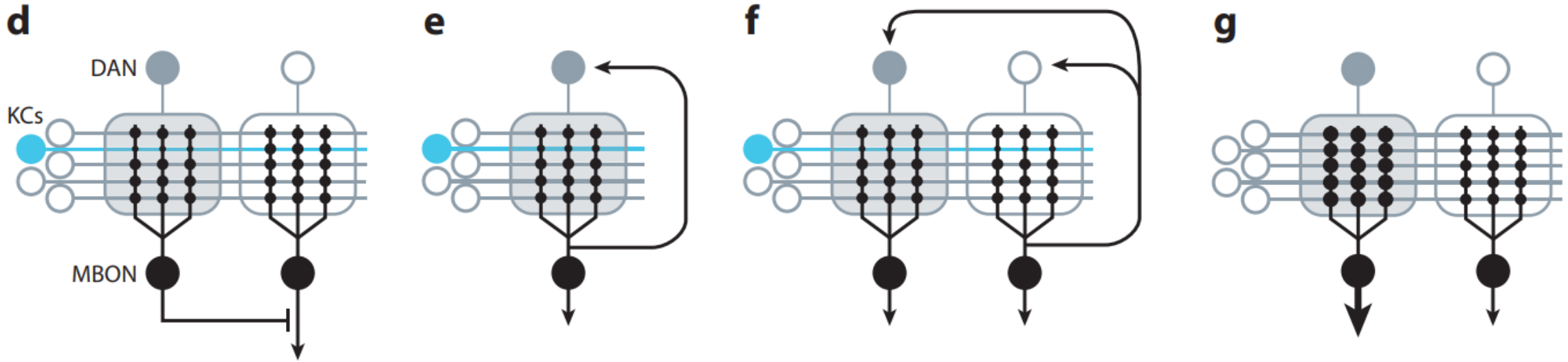
Figure 2

Valence remapping. (*a*) Mushroom body (MB) compartments. The three different Kenyon cell (KC) types (α/β, α′/β′, and γ) make contact with dopaminergic neuron (DAN)–mushroom body output neuron (MBON) modules in 15 different compartments (γ1pedc forms one compartment). Color indicates MBON neurotransmitter type. (*b*) Inverted valence maps of MBONs (*top*) and DANs (*bottom*). MBONs that signal positive valence (*blue*) are located in the compartments innervated by DANs that signal punishment (*red*). Conversely, negative-valence MBONs are paired with reward-signaling DANs. (*c*) Coincident activation of a

Conversely, negative-valence MBONs are paired with reward-signaling DANs. (*c*) Coincident activation of a KC (*light blue*) by odor and a DAN (*gray shading*) by reward depresses KC synapses onto a negative-valence MBON (*left*). This shifts the balance of overall MBON activity toward approach. Conversely, pairing odor with punishment activates DANs that project to a different compartment containing a positive-valence MBON (*right*). Depression in this compartment shifts MBON output toward avoidance.



direct excitatory input to MBONs. (*d*) Feed-forward inhibition between MBONs of opposing valence acts as a toggle switch. (*e*) Recurrent feedback of an MBON onto its cognate DAN is proposed to support persistent DAN activity that can drive associations into long-term memory. (*f*) Cross-compartment feedback to DANs allows MBON activity in one compartment to influence learning in another. This motif is involved in memory extinction and reconsolidation. (*g*) Switchboarding involves state- and context-dependent DAN activity that modulates signal transmission between KCs and MBONs, routing the same sensory input to different output channels.



Short term memory (STM) and long term memory (STM), consolidation, reconsolidation or extinction

Sort term memory (STM) and long term memory (STM)

- Aversive odor training with 12 electric shocks in 1 minute gives only STM which last for one day.
- This is Block Training. Learning is not blocked by protein synthesis inhibitors.

- Aversive odor training with spaced electric shocks every 15 seconds or more apart gives LTM which lasts up to 14 days.
- This is Spaced Training. LTM is blocked by protein synthesis inhibitors or by anaesthesis, on ice, that stops neuronal signaling. Requires translation regulation of localized transcripts at synapses. Sleep improves LTM.

- Why does LTM need the Rest Period between trainings?

Memory extinction by testing without shock.

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 3, pp. 708–712, March 1974

Conditioned Behavior in *Drosophila melanogaster*

(learning/memory/odor discrimination/color vision)

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Division of Biology, California Institute of Technology, Pasadena, Calif. 91109

Contributed by Seymour Benzer, October 25, 1973

ABSTRACT **Populations of *Drosophila* were trained by alternately exposing them to two odorants, one coupled with electric shock. On testing, the flies avoided the shock-associated odor. Pseudoconditioning, excitatory states, odor preference, sensitization, habituation, and subjective bias have been eliminated as explanations. The selective avoidance can be extinguished by retraining. All flies in the population have equal probability of expressing this behavior. Memory persists for 24 hr. Another paradigm has been developed in which flies learn to discriminate between light sources of different color.**

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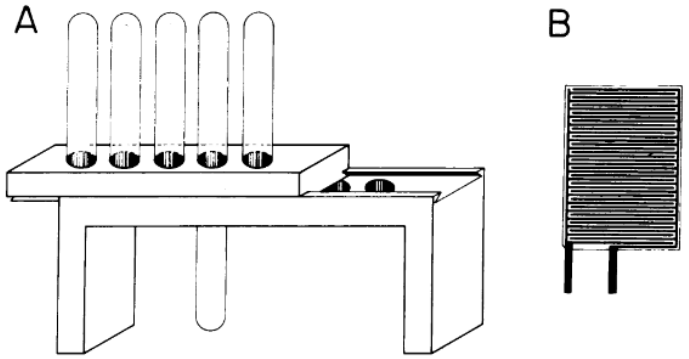


FIG. 1. (*A*) Apparatus used in the olfactory learning experiments. Two plastic blocks can be slid past each other on a dovetail joint. Holes running through each block are fitted with Teflon O-rings, to grip plastic tubes. (*B*) Printed circuit grid for shocking flies. The grid is rolled up and inserted into a plastic tube, which is plugged into the apparatus. Conductive tabs for applying voltage are bent around the tube rim to the outside.

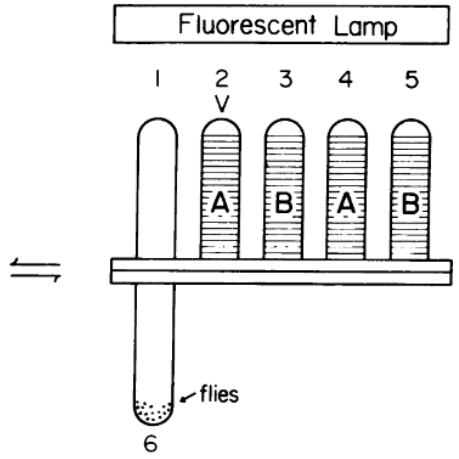


FIG. 2. Basic olfactory paradigm. Tube *1* is the rest tube, *2* and *3* are for training, *4* and *5* are for testing. Tube *6* is the start tube. *Horizontal stripes* in tubes indicate grids. *A* and *B* denote odorants 3-octanol and 4-methylcyclohexanol, respectively. *V* indicates voltage on the grid. See *text* for training and testing sequences.

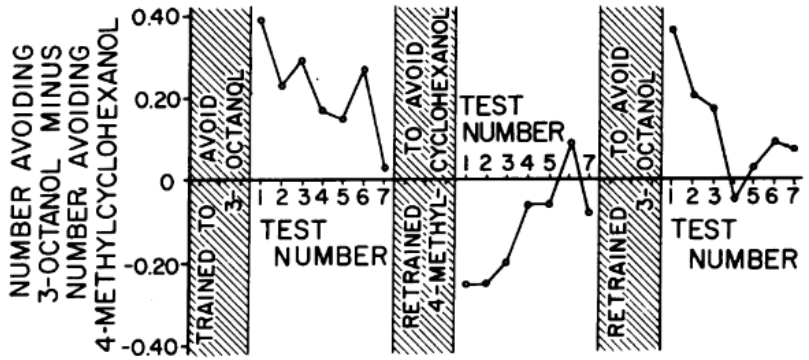


FIG. 3. Extinction and reversal of the learned response. A population of 36 flies was trained to avoid 3-octanol, then tested repeatedly without reinforcement. They were reverse-trained to avoid 4-methylcyclohexanol and retested, then reverse-trained and tested again.

In the basic paradigm (Fig. 2) tube 1 is a "rest" tube with holes at the end to allow odor to escape. Tubes 2–5 contain grids with odorants: tubes 2 and 4 each have 3-octanol on their grids; tubes 3 and 5 have 4-methylcyclohexanol. Tubes 2 and 3 are used for training, tubes 4 and 5 for testing. Voltage is applied to tube 2 only. The use of separate tubes for training and testing removes the flies from any odors they may have left on the grids during training, so that during testing the chemical odorants are the only possible cues for selective avoidance.

For training, the sequence of runs was: rest tube (60 sec), tube 2 (15 sec), rest tube (60 sec), tube 3 (15 sec). This cycle was repeated three times. (A tendency to avoid tube 2 was already evident by the second cycle.) The flies were then tested in the same sequence with tubes 4 and 5 instead of 2 and 3. The number of flies avoiding the grid on each run was counted visually. More flies avoided tube 4 than tube 5. Tube 4 contained 3-octanol, which had been presented simultaneously with shock during training.

LTM consolidation. Recurrent connections from MBONS to DANS may alter the synaptic microcircuit at the odor cue Kc cell to hold the learned information until protein synthesis consolidates the memory.

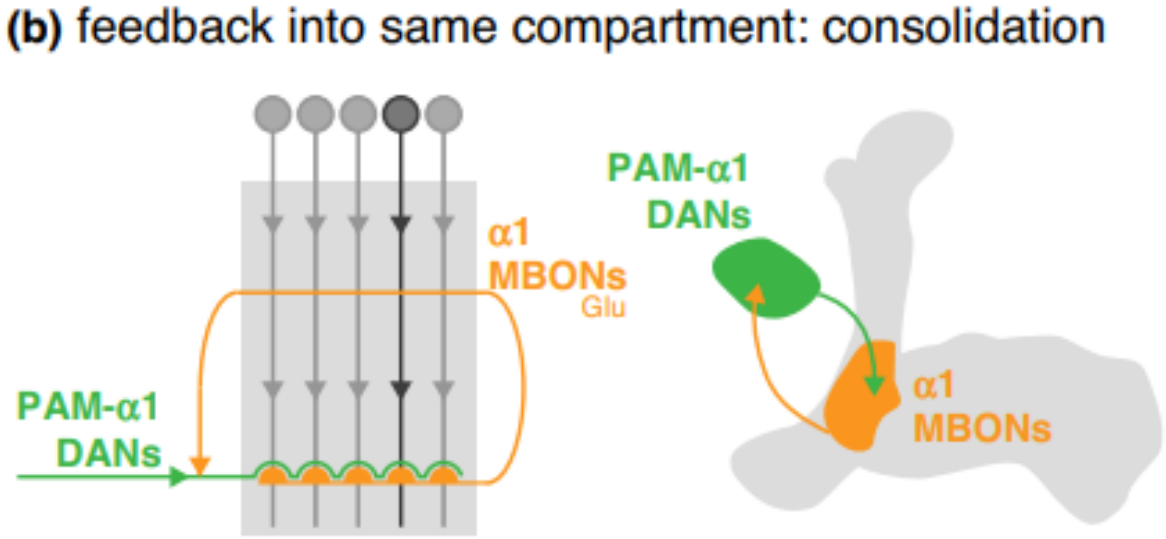
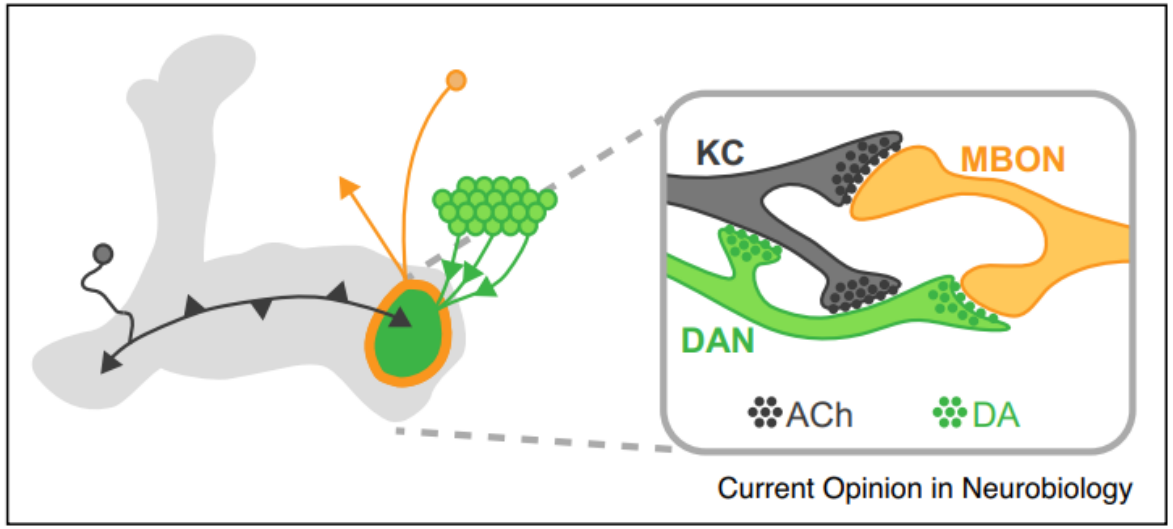
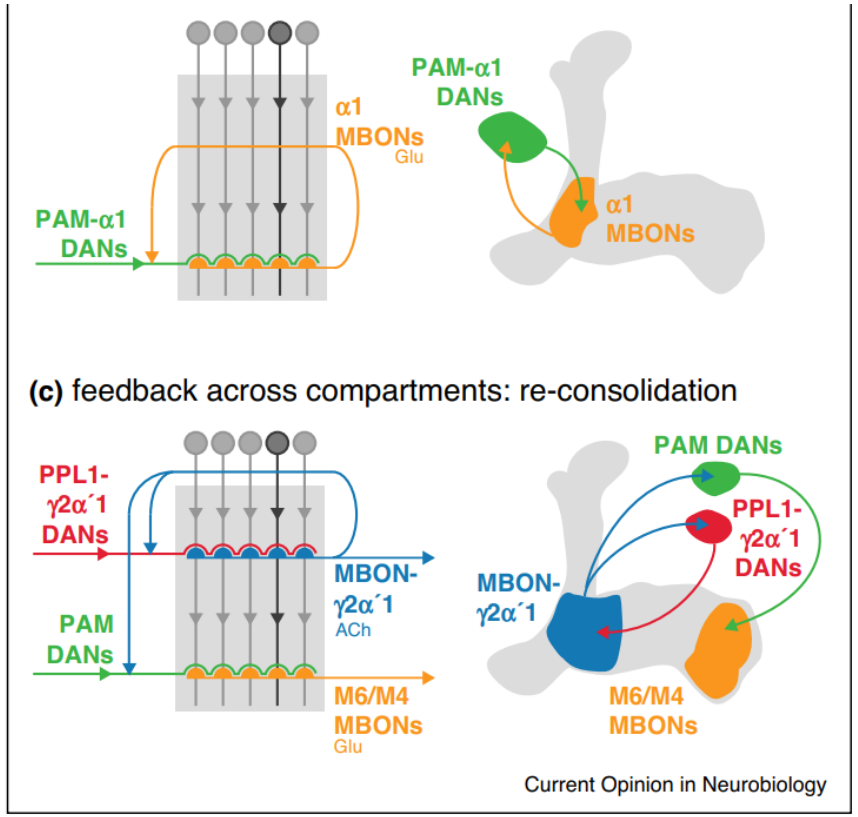


Figure 4



KCs, MBONs and DANs form microcircuits within a compartment [5••,7••]. KCs (black) form cholinergic excitatory connections with MBONs (orange) and also synapse onto DANs (green). KC to DAN connections appear to be excitatory and may modulate the dopaminergic reinforcement signal within a compartment to influence learning [60]. DANs synapse onto KCs and MBONs. Activation of DANs seems to lead to a slow but direct activation of MBONs which potentially allows a local excitatory feedback loop between KCs, DANs and MBONs [5••].

Memory reconsolidation or extinction involve interactions between MB compartments



Feed-forward, feed-back and feed-across networks in the mushroom body. **(a)** Feed forward inhibition regulates state dependent expression of food memory retrieval. Hunger state controls odor-driven behavior by relaying hunger-dependent dNPF signaling (purple) through the

comprised of the MP1 DANs [21] and a pair of GABAer-gic neurons, called MVP2 (or MBON-γ1pedc > αβ), that exert feedforward inhibition across the MBON network [12••] (Figure 3a). In hungry flies, inhibition from MVP2

PPL1-MP1 DANs (red) and the GABAergic MVP2 neuron (blue). The MVP2 neuron feeds forward inhibition to the M4/M6 group of glutamatergic MBONs (orange). In a satiated fly, approach behavior is counterbalanced by the avoidance-promoting M4/M6 MBONs, which are not inhibited by MVP2. In a hungry fly, the MVP2 neuron is active and inhibits the M4/M6 MBONs, reducing avoidance and facilitating approach behavior. **(b)** A closed feedback loop involving the MBON from the α1 compartment, the αβ Kenyon cells and the DANs innervating the α1 compartment has been proposed to stabilize memory after learning. Blocking any of these neurons immediately after sugar reward learning cripples behavioral approach measured 24 h later [50•], although a functional connection between the glutamatergic α1 MBON and α1 DANs has not been demonstrated. **(c)** A feedback and feed-across recurrency is crucial for memory reconsolidation. Experiencing training-related cues can switch stable memories back into a labile state and reconsolidation is then required for the memory to return to a persistent condition [9••]. The reconsolidation process requires the activity of the cholinergic MBON-γ2α′1 which in turn recruits two different sets of dopaminergic neurons: the PPL1-γ2α′1 DANs, which are required during memory retrieval, and a group of PAM-DANs which innervate different compartments and are required in the period following retrieval. These cholinergic MBON-DAN connections are excitatory [9••].

The neuronal architecture of the mushroom body provides a logic for associative learning

Yoshinori Aso[1]*, Daisuke Hattori[2], Yang Yu[1], Rebecca M Johnston[1], Nirmala A Iyer[1], Teri-TB Ngo[1], Heather Dionne[1], LF Abbott[3,4], Richard Axel[2,3,7], Hiromu Tanimoto[5,6], Gerald M Rubin[1]*

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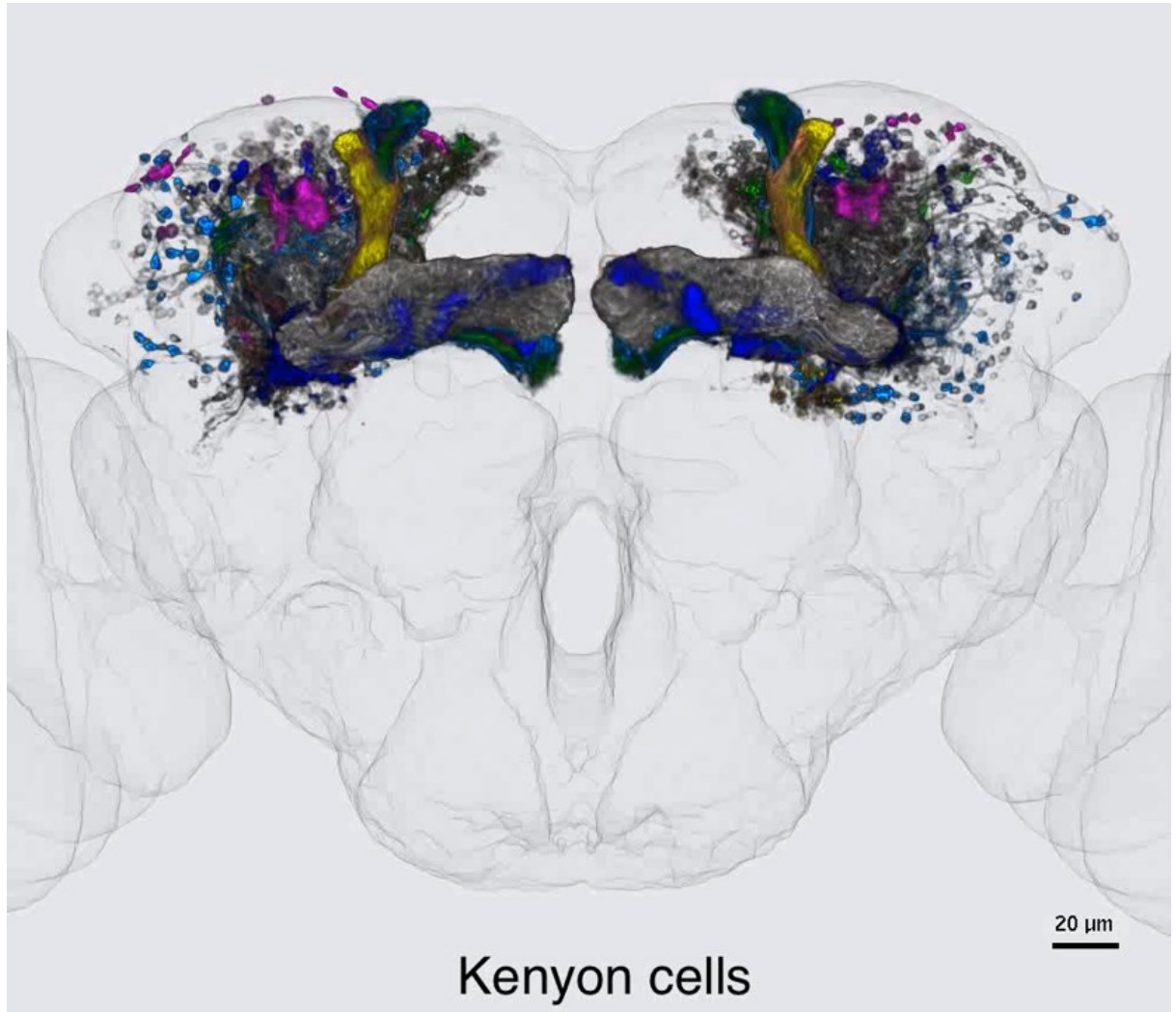
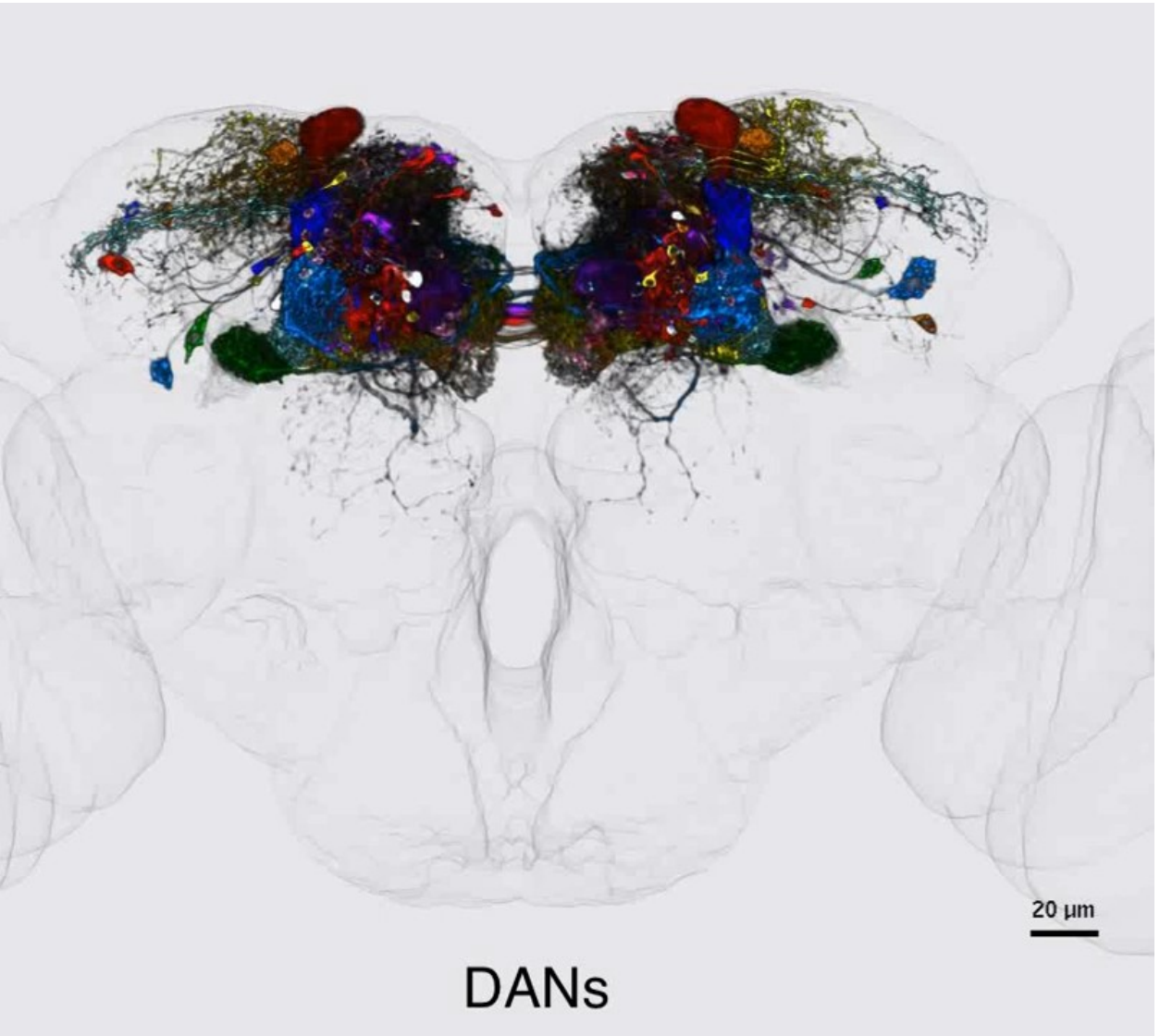
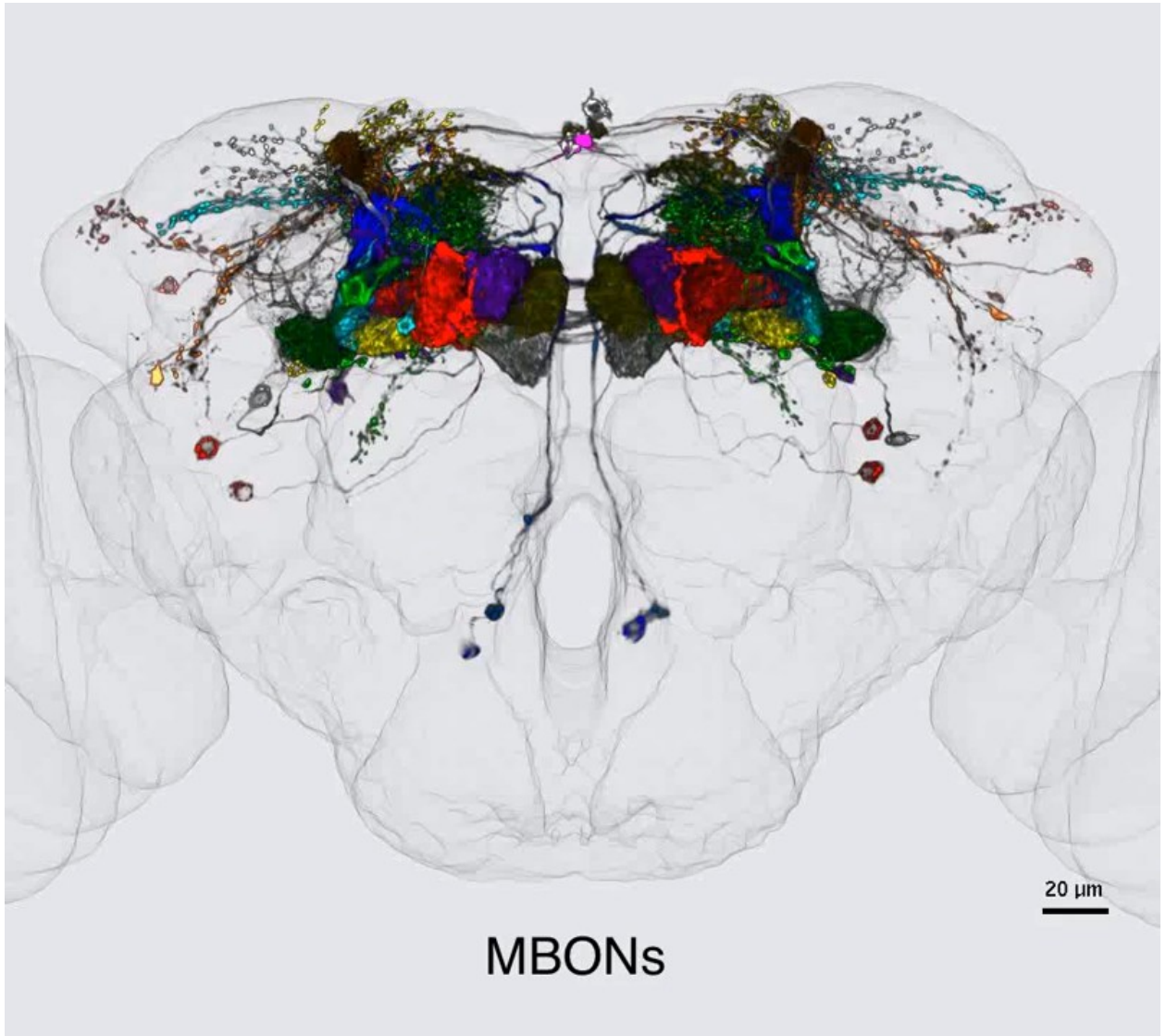
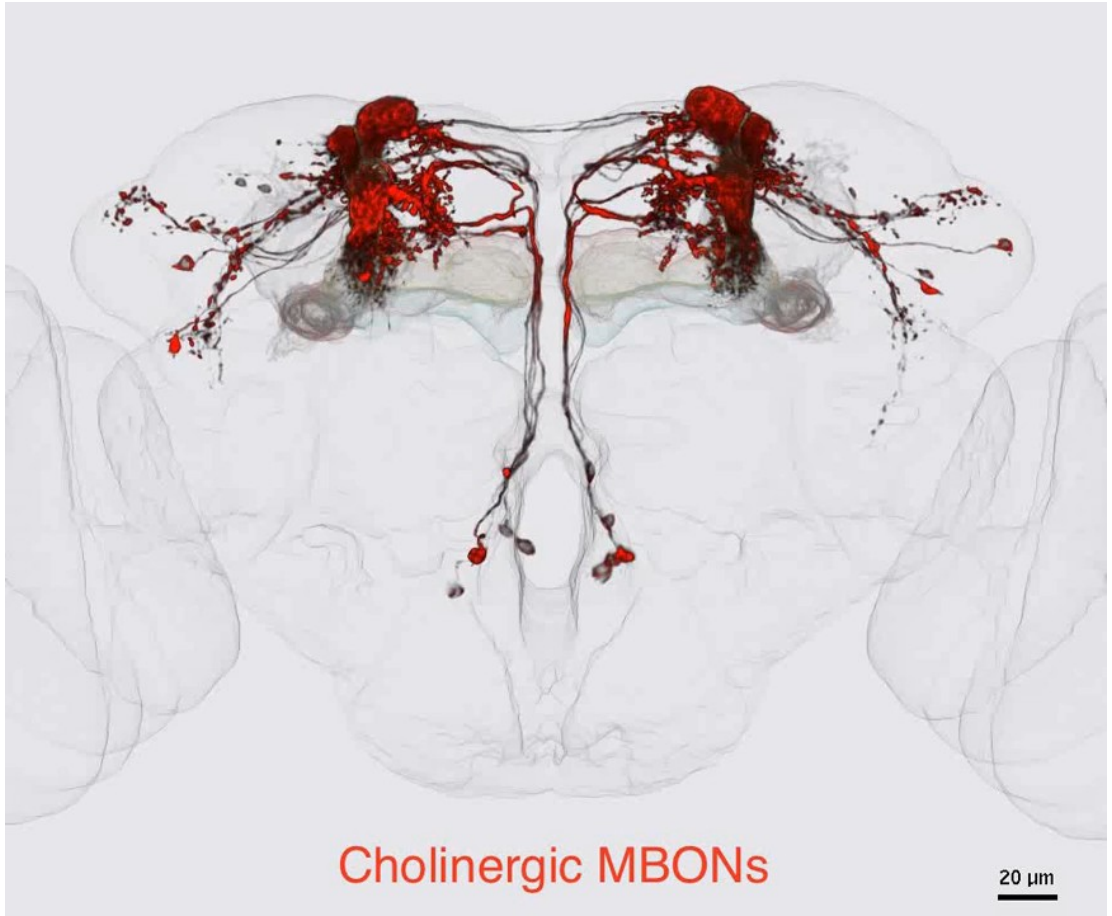


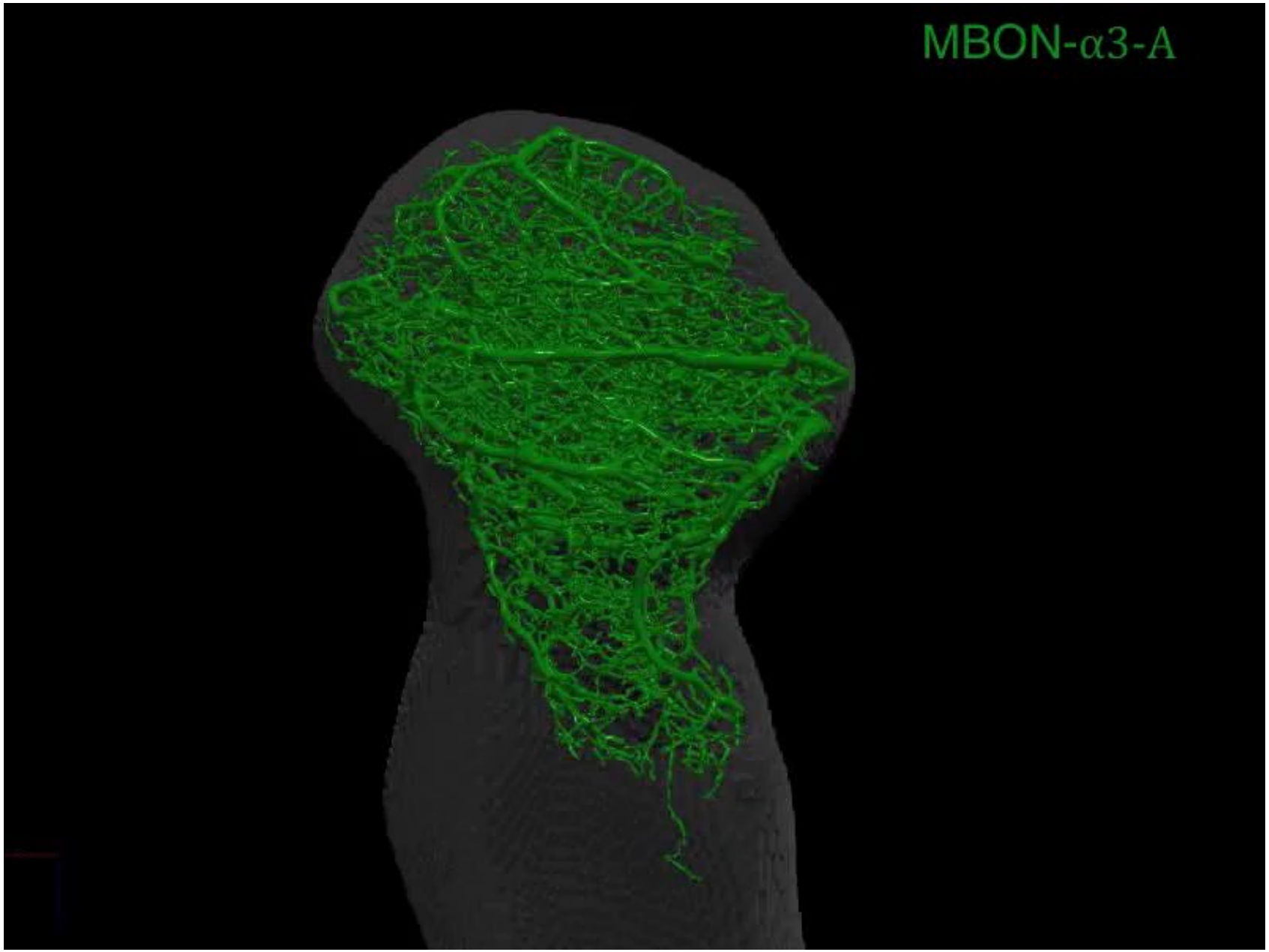


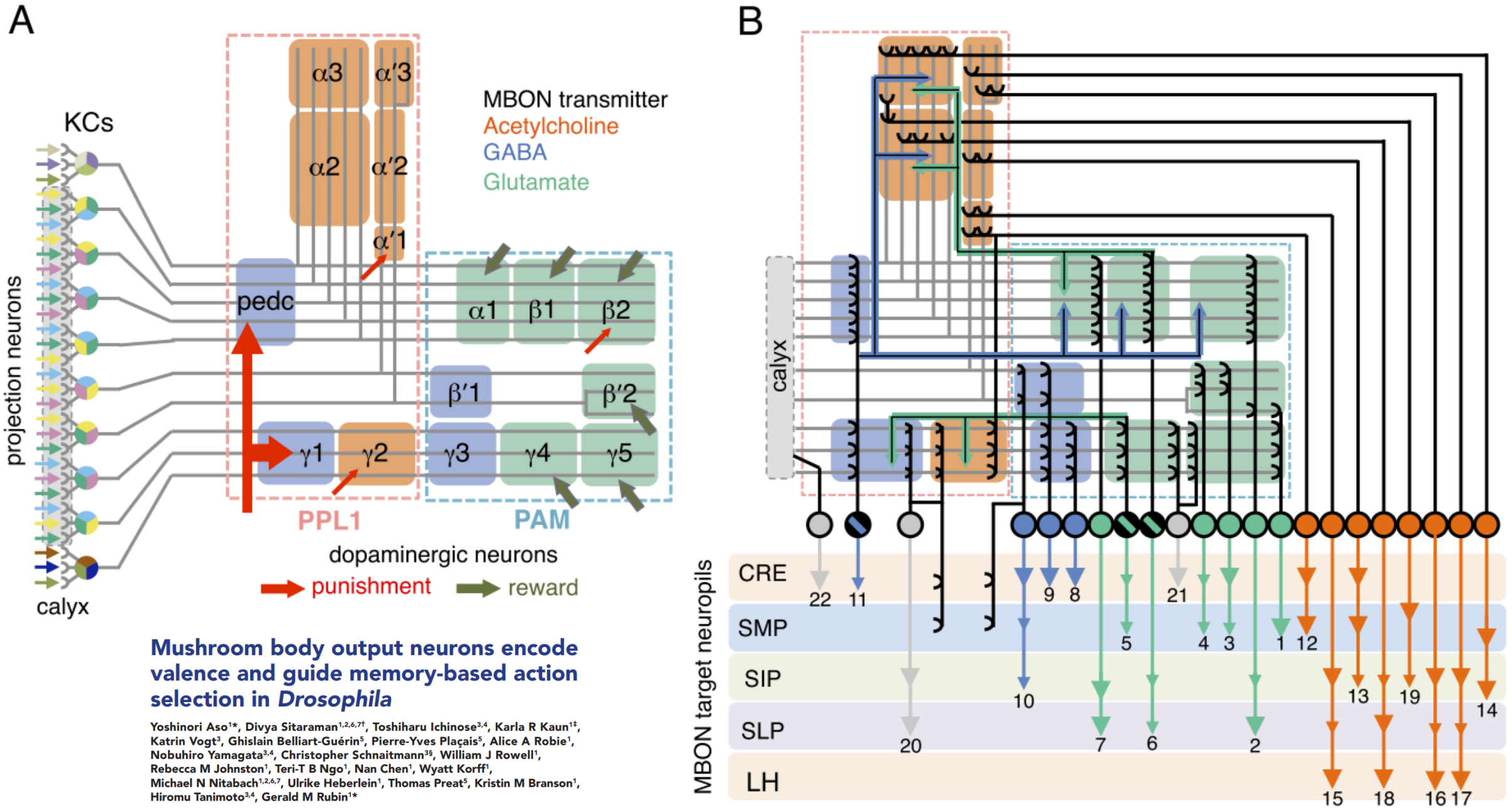
DANs



MBONs


Cholinergic MBONs
20 µm


MBON-α3-A

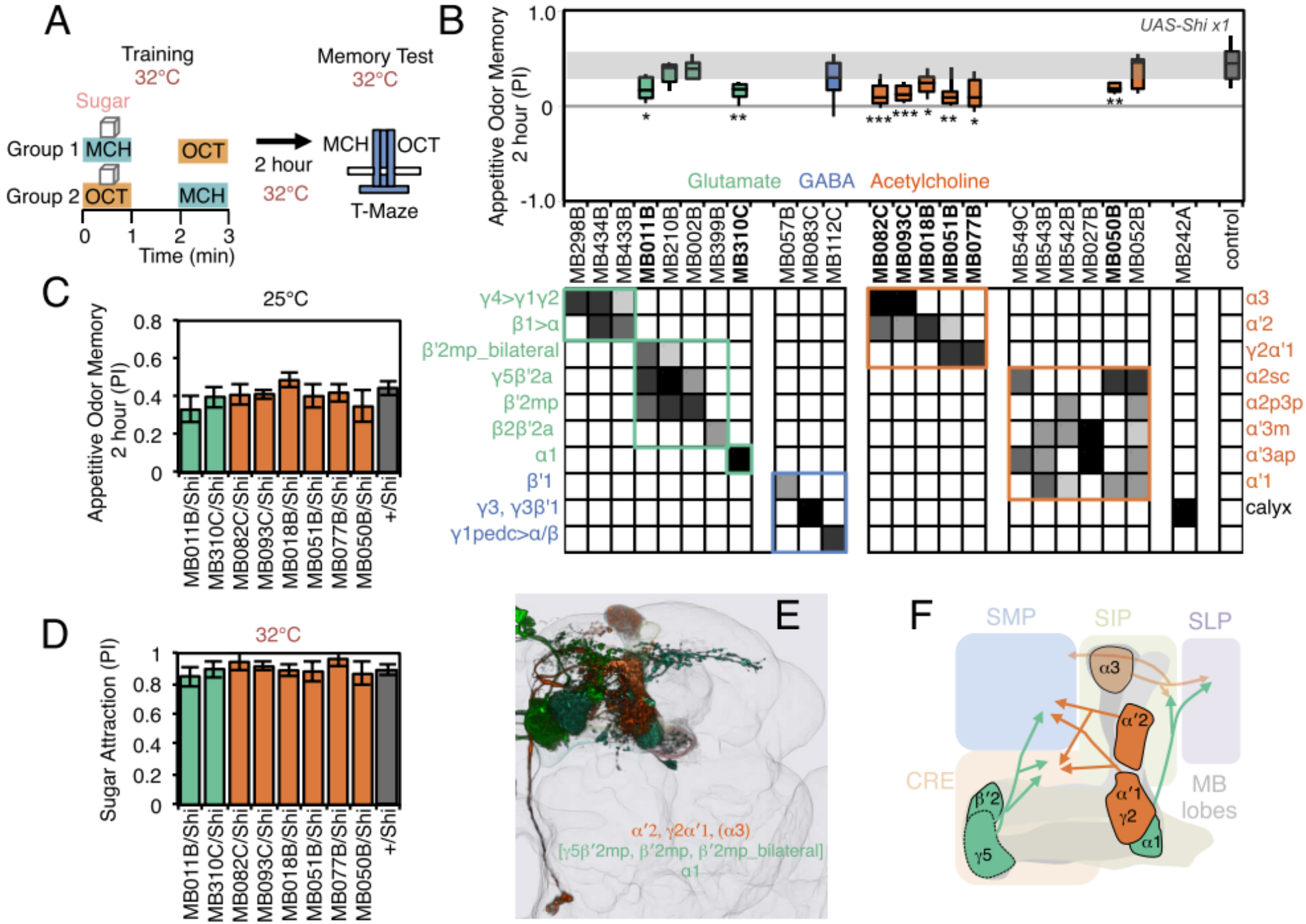


Mushroom body output neurons encode valence and guide memory-based action selection in *Drosophila*

Yoshinori Aso[1]*, Divya Sitaraman[1,2,6,7†], Toshiharu Ichinose[3,4], Karla R Kaun[1‡], Katrin Vogt[3], Ghislain Belliart-Guérin[5], Pierre-Yves Plaçais[5], Alice A Robie[1], Nobuhiro Yamagata[3,4], Christopher Schnaitmann[3§], William J Rowell[1], Rebecca M Johnston[1], Teri-T B Ngo[1], Nan Chen[1], Wyatt Korff[1], Michael N Nitabach[1,2,6,7], Ulrike Heberlein[1], Thomas Preat[5], Kristin M Branson[1], Hiromu Tanimoto[3,4], Gerald M Rubin[1]*

[1]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States; [2]Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, United States; [3]Max Planck Institute of Neurobiology, Martinsried, Germany; [4]Graduate

Figure 1. Circuit diagrams of the mushroom body. (**A**) The innervation patterns of extrinsic neurons define 15 compartments in the MB lobes and one compartment in the core of distal pedunculus (pedc); the compartments are represented by rectangles that are color-coded based on the neurotransmitter used by the mushroom body output neurons (MBONs) having dendrites in that compartment (green, glutamate; blue, GABA; orange, acetylcholine). Projection neurons (far left, colored arrows) from the antennal lobe convey olfactory sensory information to the MB calyx where they synapse on the dendrites of Kenyon cells (KCs). The parallel axon fibers of the KCs (gray lines) form the lobes (α/β, α′/β′ and γ) where KCs terminate onto the dendrites of the MBONs. Each of the seven types of KCs innervates a specific layer within a given lobe. The dendrites of individual MBON types and the terminals of dopaminergic neurons (DANs) intersect the longitudinal axis of KC axon-bundles in specific compartments along the lobes. MBONs using the same transmitter are spatially co-localized in the lobes (See ***Figure 1B***). Innervation areas of PPL1 and PAM cluster DANs axons in the MB lobes are indicated by the rectangles outlined in dashed lines. Activation of subsets of DANs can convey punishment or reward, respectively inducing aversive or appetitive memory when activation is paired with odor presentation. The size of arrows indicates magnitude of memory induced by DAN activation. See text for references. (**B**) Schematic representation of the 21 cell types of MBONs in the lobes and one cell type of MBON in the calyx based on the data presented in the accompanying manuscript (***Aso et al., 2014***): circles, cell bodies; semicircles, dendrites; arrowheads, axon terminals; color-coding is by neurotransmitter as in panel (**A**) Three MBON cell-types (GABAergic MBON-γ1pedc>α/β, glutamatergic MBON-γ4>γ1γ2 and MBON-β1>α; marked as 11, 5 and 6 respectively) send axons into the MB lobes. Axons of MBON-γ4>γ1γ2 project from γ4 to γ1 and γ2, and thus have the potential to affect activity of MBON-γ1pedc>α/β. From γ1, the axon of MBON-γ1pedc>α/β projects to compartments in the α/β lobes including β1, where dendrites of MBON-β1>α arborize. Axons of both MBON-γ1pedc>α/β and MBON-β1>α project to the compartments in the α lobe. Therefore activity of MBONs in the α lobe can be regulated by these layered inter-compartmental connections. These three types of MBONs (11, 5 and 6) do not project back to their own dendrites. Therefore, the organization of the MBONs can be viewed as forming a multilayered feed-forward network (***Aso et al., 2014***). MBONs project to a small number of brain areas: the crepine (CRE; a region surrounding the horizontal/medial lobes), the superior medial protocerebrum (SMP), superior intermediate protocerebrum (SIP) and superior lateral protocerebrum (SLP) and the lateral horn (LH). The size of the arrowhead reflects the relative number of termini in each area. The MBONs are numbered and listed in ***Table 1***. See the accompanying manuscript (***Aso et al., 2014***) and ***Table 1*** for details.
DOI: 10.7554/eLife.04580.003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Glutamate GABA Acetylcholine
MB298B MB434B MB433B MB011B MB210B MB002B MB399B MB310C MB057B MB083C MB112C MB082C MB093C MB018B MB051B MB077B MB549C MB543B MB542B MB027B MB050B MB052B MB242A control
γ4>γ1γ2
β1>α
β'2mp_bilateral
γ5β'2a
β'2mp
β2β'2a
α1
β'1
γ3, γ3β'1
γ1pedc>α/β
α3
α'2
γ2α'1
α2sc
α2p3p
α'3m
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25°C
Appetitive Odor Memory
2 hour (PI)
0.8
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MB310C/Shi
MB082C/Shi
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MB082C/Shi
MB093C/Shi
MB018B/Shi
MB051B/Shi
MB077B/Shi
MB050B/Shi
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E
α′2, γ2α′1, (α3)
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α1
F
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SIP
SLP
α3
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CRE
β′2
γ5
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MB
lobes

Figure 7. Requirement of MBONs for 2-hr appetitive odor memory. (**A**) Flies were trained and tested in a similar way as in ***Figure 6A***, except that flies were starved for 28–40 hr prior to experiments and trained with a reward consisting of a tube covered with sugar absorbed filter paper. (**B**) Results of secondary screening of MBONs for 2-hr appetitive memory using *UAS-Shi x1*. MBONs have been grouped by neurotransmitter and color-coded. The bottom and top of each box represents the first and third quartile, and the horizontal line dividing the box is the median. The whiskers represent the 10th and 90th percentiles. The gray rectangle spanning the horizontal axis indicates the interquartile range of the control. Statistical tests are described in methods: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The relative expression levels produced by the split-GAL4 driver lines in each cell type (indicated by the gray scale) are based on imaging with *pJFRC2-10XUAS-IVS-mCD8::GFP* (*VK00005*). Images of the expression patterns of selected drivers are shown in ***Figure 7—figure supplement 1***. MB082C and MB093C label the cholinergic MBON-α3; however, these lines also show weak labeling in MBON-α′2. As blocking MBON-α′2 alone also gave a significant phenotype (MB018B/Shi), we cannot rule out the formal possibility that the phenotype observed with MB082C and MB093C results from blocking MBON-α′2. MB050B labels MBON-α2 and MBON-α′1; however, because MB052B, which labels the same cell types in addition to others, did not give a phenotype, the requirement for these cell types is not resolved. (**C**) 2 hr appetitive odor memory at permissive temperature. (**D**) Sugar attraction in untrained flies at restrictive temperature. (**E**) Rendering of MBONs with outline of MB lobes and brain. MBONs grouped by square brackets represent cases where the available set of driver lines do not allow assigning an effect to a single cell type, but only to that set of MBONs. MBONs in parentheses represent cases where the data implicating them are only suggestive. (**F**) Diagram of MBONs for appetitive odor memory. MBONs are shown in lighter colors when data implicating them are only suggestive.

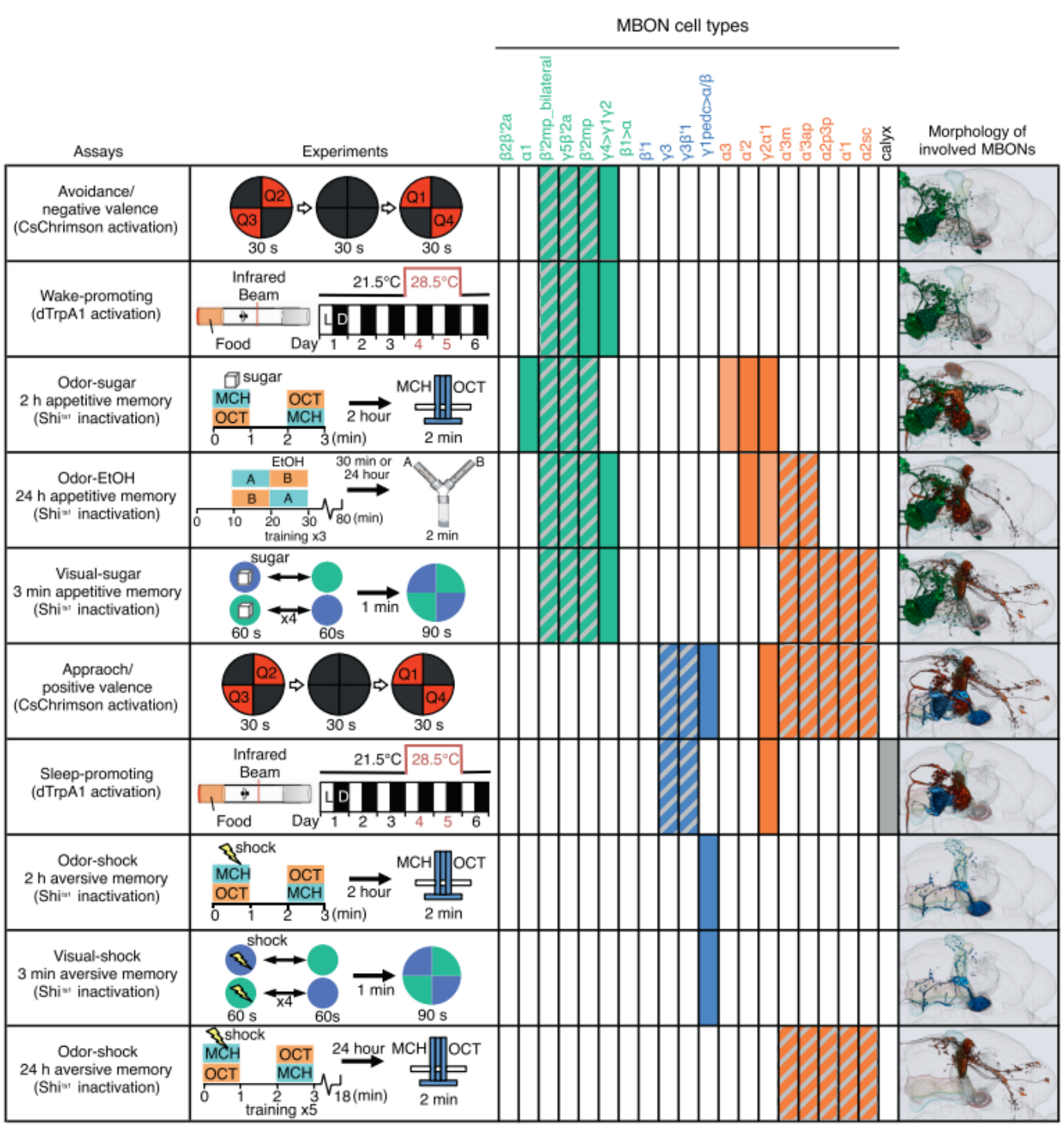
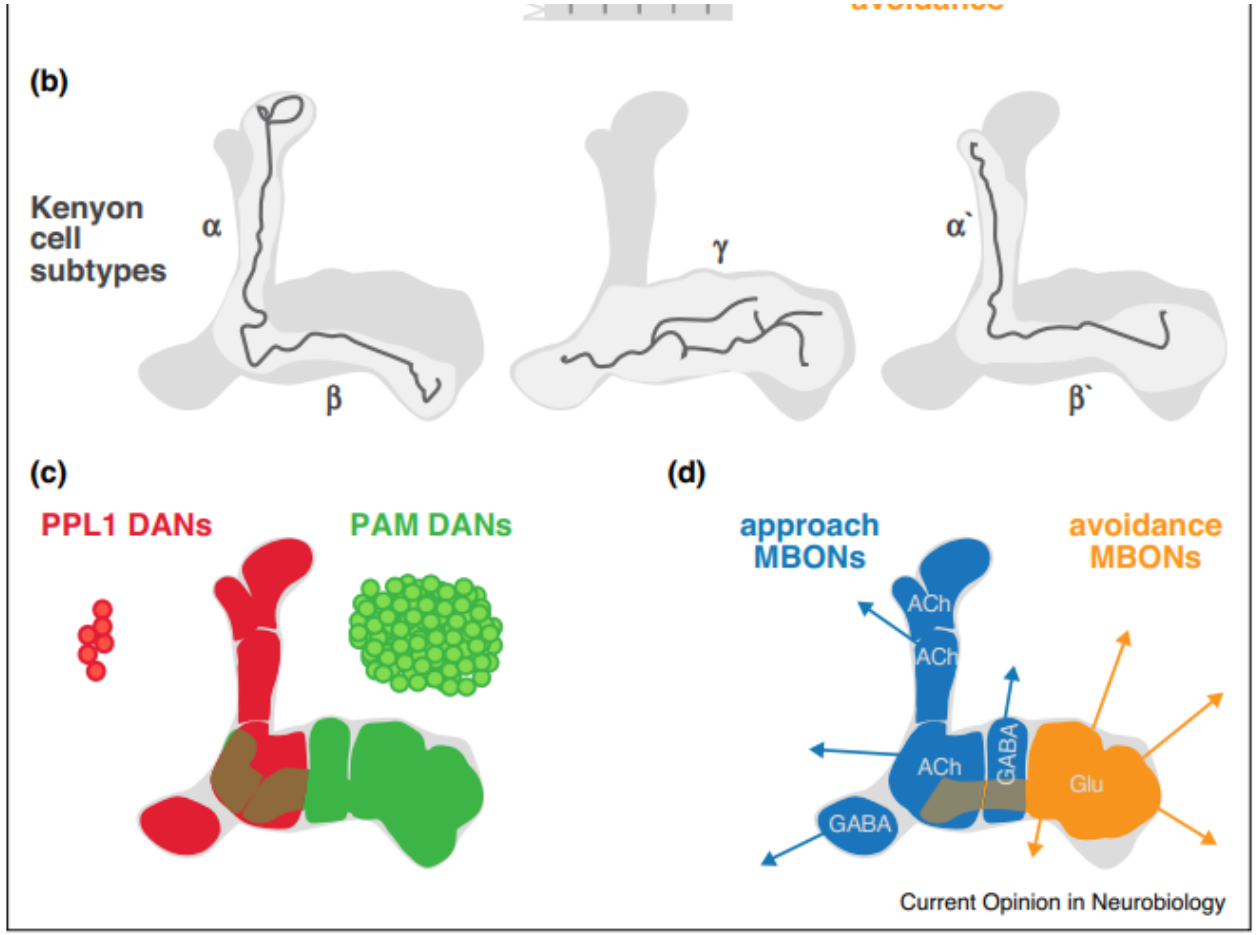


Figure 13. A map of MBON functions. A matrix summarizing the M
matrix representing MBON cell types, solid colors were assigned v
Hatched cells indicate cases where we observed a significant beha
cases, our data only allow us to assign the phenotypic effect to
suggested the involvement of a cell type, but was not conclusive.
phenotypes reflect the results of activating MBONs; the other a
glutamatergic MBONs repelled flies and promoted wakefulness, v
sleep. In general, more cell types were involved in appetitive memo
MBONs were required for all three appetitive memory assays. The
memory in an assay protocol that did not distinguish between forn
cluster MBONs.

The mushroom body is the center for associative learning. **(a)** Sensory cues are represented as activity in sparse populations of cholinergic Kenyon cells (KCs, grey). KCs send their neurites into the lobes of the mushroom body (light grey background), where they make *en passant* synapses with the output neurons (MBONs). Mushroom body-innervating dopaminergic neurons (DANs) provide the reinforcement signal during aversive and appetitive associative learning. **(b)** KCs are organized into three subtypes which make up the lobes of the mushroom body neuropil (individual representative KCs shown in dark grey). **(c)** The presynaptic fields of the different DAN classes tile the mushroom body into non-overlapping compartments. **(d)** The dendritic tufts of distinct MBONs match the compartmentalization of the DAN teminals. Aversively reinforcing DANs in the paired posterior lateral 1 (PPL1) cluster (red) overlap with approach-promoting MBONs (blue), while DANs of the protocerebral anterior medial (PAM) cluster, which are largely appetitively reinforcing (green), overlap with avoidance-promoting MBONs (orange). The transmitters used by each class of neuron is noted: ACh, acetylcholine; DA, dopamine; GABA, γ-aminobutiryc acid; Glu, glutamate.



Internal states affect types of learning

- Hungry. Thirst. Well fed.
- Courting. Mated. Frustrated.
- Defeated. Depressed.
- Alcoholic. Drug-addicted.
- Innate immunity and sleep.
- Sociable. Imitating others.

Do the right thing: neural network mechanisms of memory formation, expression and update in *Drosophila*

Paola Cognigni, Johannes Felsenberg and Scott Waddell

When animals learn, plasticity in brain networks that respond to specific cues results in a change in the behavior that these cues elicit. Individual network components in the mushroom bodies of the fruit fly *Drosophila melanogaster* represent cues, learning signals and behavioral outcomes of learned experience. Recent findings have highlighted the importance of dopamine-driven plasticity and activity in feedback and feedforward connections, between various elements of the mushroom body neural network. These computational motifs have been shown to be crucial for long term olfactory memory consolidation, integration of internal states, re-evaluation and updating of learned information. The often recurrent circuit anatomy and a prolonged requirement for activity in parts of these underlying networks, suggest that self-sustained and precisely timed activity is a fundamental feature of network computations in the insect brain. Together these processes allow flies to continuously adjust the content of their learned knowledge and direct their behavior in a way that best represents learned expectations and serves their most pressing current needs.

experience: identifying the nature of this plasticity and how it yields the changes in behavior that arise after training is a core goal of neuroscience.

Drosophila has been used as a model for associative learning for nearly half a century [1]: from the anatomical structures [2], to the cell types [3,4] and their precise connectivity [5••,6,7••], the physical substrates of memory in flies are being elucidated to finer and finer detail [8,9••,10••,11,12••,13–15]. Anatomy and function have been linked by experiments in which specific neurons are targeted genetically and their firing activity or synaptic release artificially altered, using temperature or light to control the timing of intervention. Within an associative learning paradigm, a neuron can therefore be prevented from functioning: if learning is disrupted, that neuron is likely to play a role. Conversely, if the artificial activation of the neuron can replace some component of the training paradigm, its function in the network is likely to represent that particular component [16–24]. In parallel with these

An integrative sensor of body states: how the mushroom body modulates behavior depending on physiological context

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The brain constantly compares past and present experiences to predict the future, thereby enabling instantaneous and future behavioral adjustments. Integration of external information with the animal's current internal needs and behavioral state represents a key challenge of the nervous system. Recent advancements in dissecting the function of the *Drosophila* mushroom body (MB) at the single-cell level have uncovered its three-layered logic and parallel systems conveying positive and negative values during associative learning. This review explores a lesser-known role of the MB in detecting and integrating body states such as hunger, thirst, and sleep, ultimately modulating motivation and sensory-driven decisions based on the physiological state of the fly. State-dependent signals predominantly affect the activity of modulatory MB input neurons (dopaminergic, serotoninergic, and octopaminergic), but also induce plastic changes directly at the level of the MB intrinsic and output neurons. Thus, the MB emerges as a tightly regulated relay station in the insect brain, orchestrating neuroadaptations due to current internal and behavioral states leading to short- but also long-lasting changes in behavior. While these adaptations are crucial to ensure fitness and survival, recent findings also underscore how circuit motifs in the MB may reflect fundamental design principles that contribute to maladaptive behaviors such as addiction or depression-like symptoms.

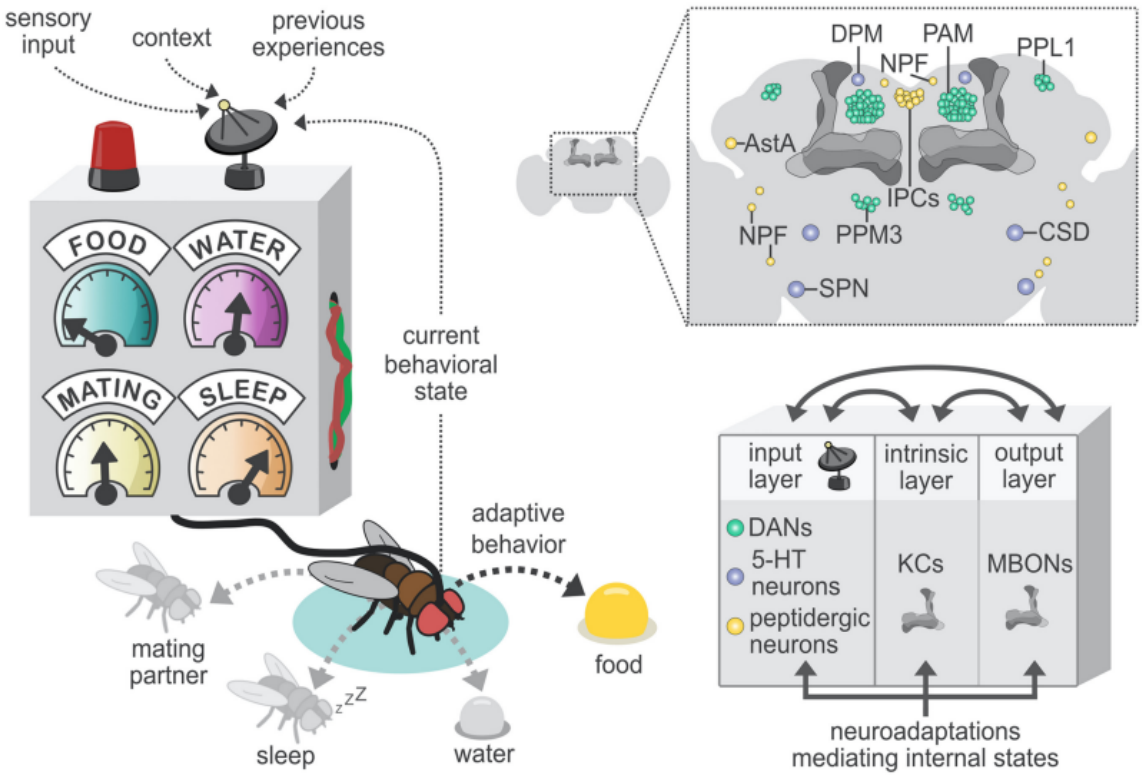
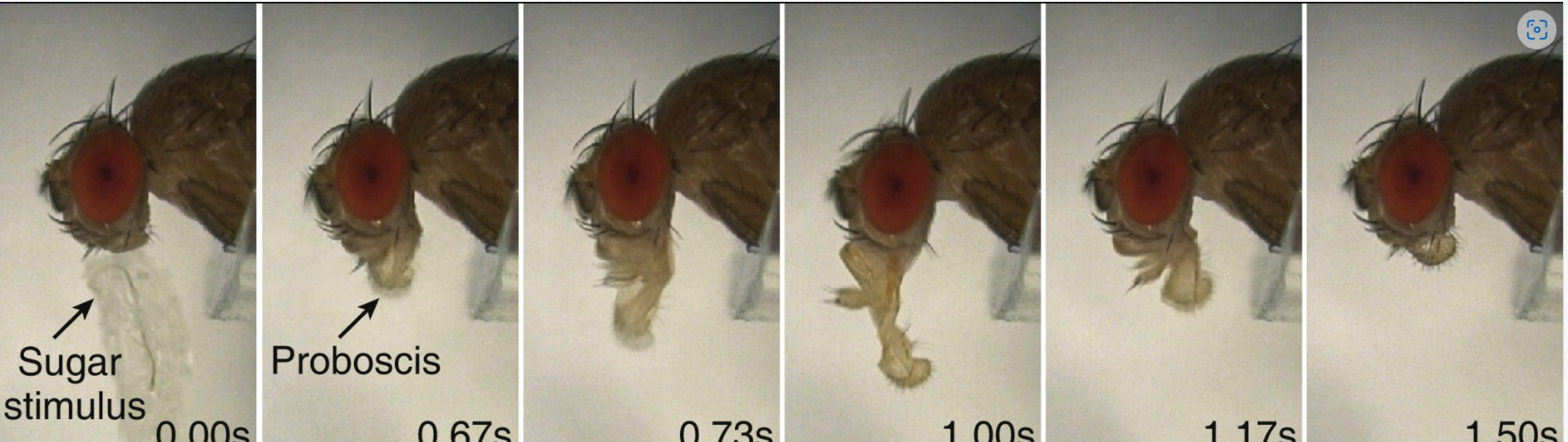


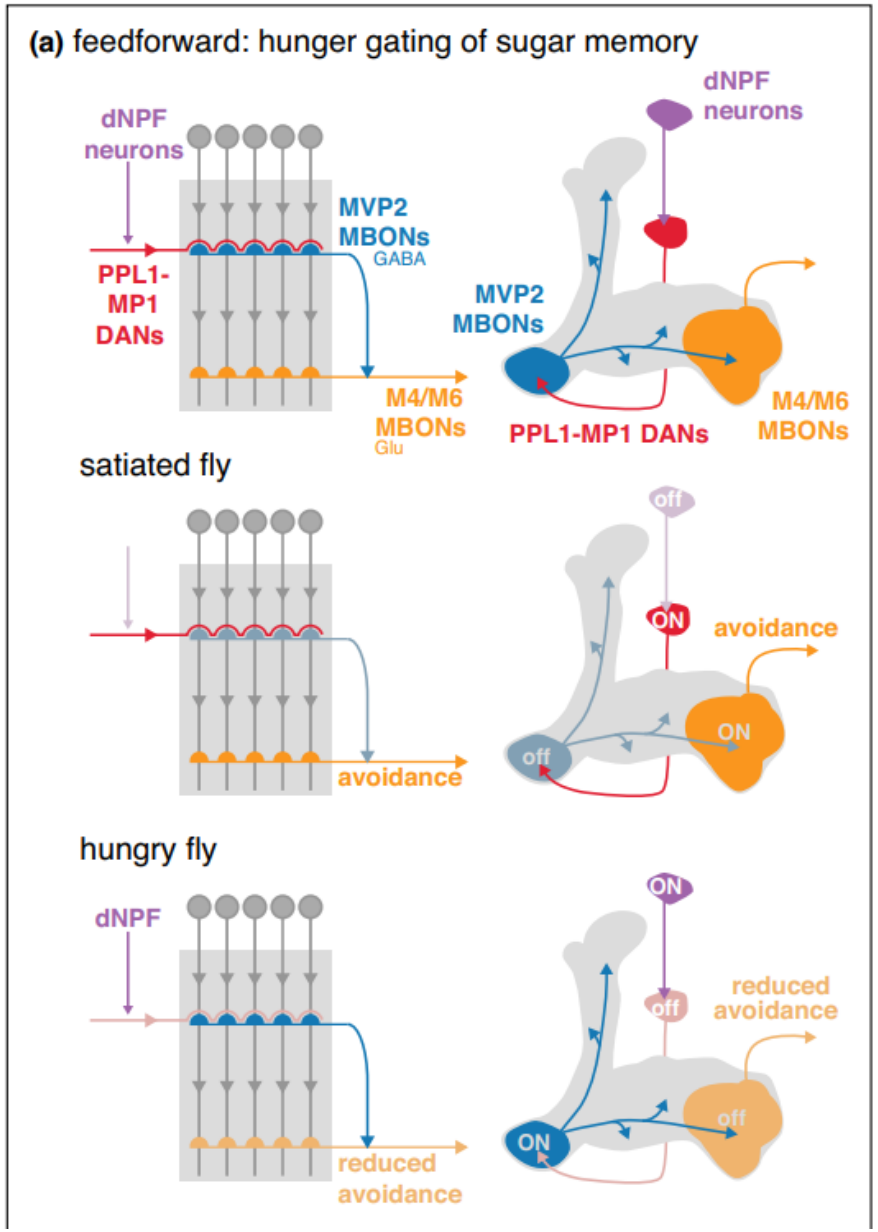
Figure 1. The MB as a panel control of body states. (*Left*) The MB integrates sensory information about the environment and the current context and behavioral state to regulate sensory valence according to the physiological needs of the fly in a moment-to-moment basis. As different internal states are being signaled in common or interconnected circuitries, this supports a tight regulation by body needs of the expression of the behavior that is most adaptive at each moment (e.g., eat and remember associated cues when starving). (*Right*, *top* and *bottom*) Information about the environment and behavioral state is sensed and signaled to the intrinsic and output layers of the MB (comprised of KCs and MB MBONs, respectively) through the input layer. The input layer contains a broad array of circuitries distributed all over the brain, including dopaminergic (DANs; green), serotonergic (5-HT; purple), and peptidergic (such as allatostatin A [AstA] and neuropeptide-F [NPF]; yellow) populations. The full name of these neural populations can be found in the text. (*Bottom right*) Short-lived and long-lasting plastic changes at the level of the three MB layers allow the fly to adapt its behavior instantaneously and in the future based on previous experience. Recurrent connectivity between the input, intrinsic, and output circuitries further potentiates plasticity across MB layers.

Internal states and types of learning

- Hungry. - Approach food odors. Overcome aversion to some odors or to bitter tastes. Flies should be hungry for Appetitive Taste or Odor training.

- Thirsty. - Approach food and water.



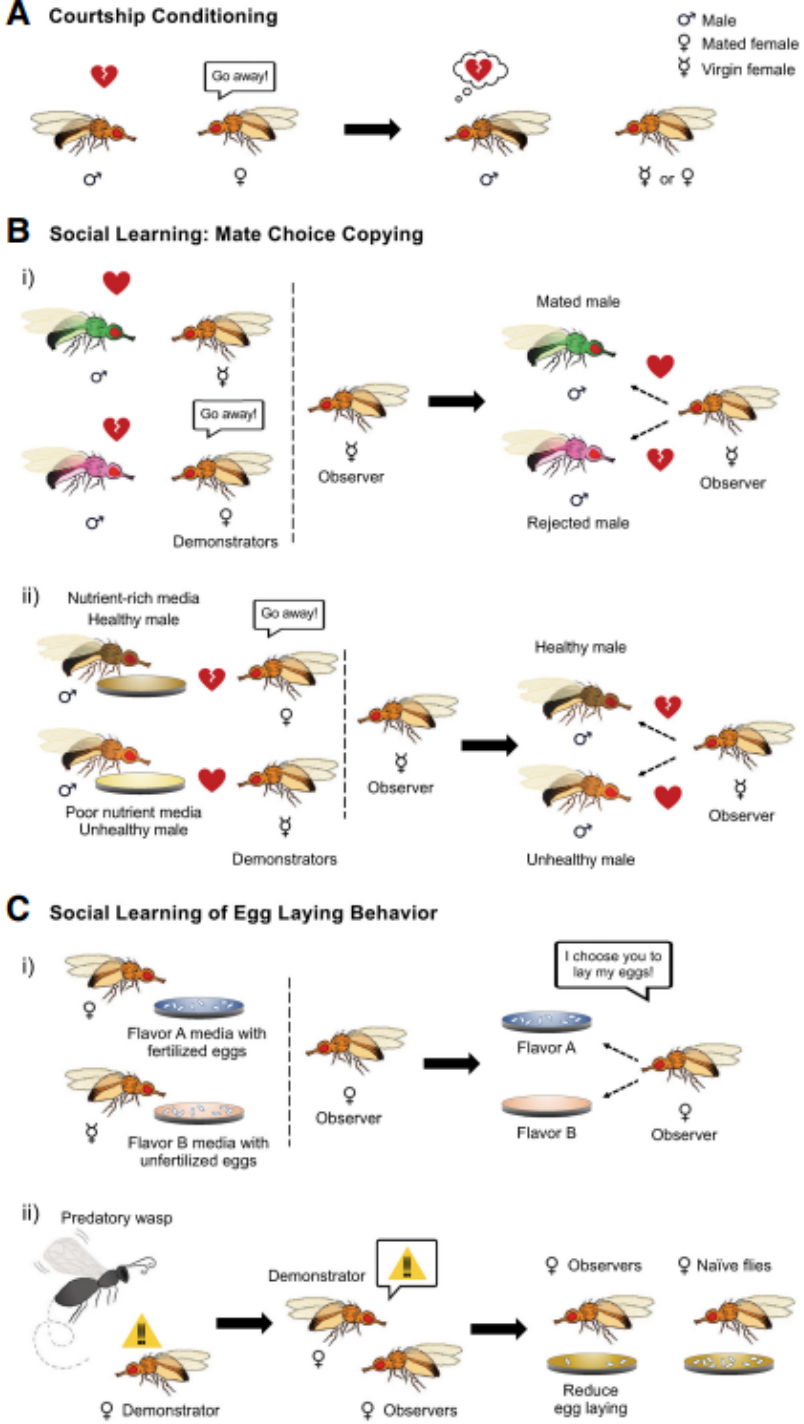


Feed-forward, feed-back and feed-across networks in the mushroom body. **(a)** Feed forward inhibition regulates state dependent expression of food memory retrieval. Hunger state controls odor-driven behavior by relaying hunger-dependent dNPF signaling (purple) through the PPL1-MP1 DANs (red) and the GABAergic MVP2 neuron (blue). The MVP2 neuron feeds forward inhibition to the M4/M6 group of glutamatergic MBONs (orange). In a satiated fly, approach behavior is counterbalanced by the avoidance-promoting M4/M6 MBONs, which are not inhibited by MVP2. In a hungry fly, the MVP2 neuron is active and inhibits the M4/M6 MBONs, reducing avoidance and facilitating approach behavior. **(b)** A closed feedback loop involving the MBON from the α1 compartment, the αβ Kenyon cells and the DANs innervating the α1 compartment has been proposed to stabilize memory after learning. Blocking any of these neurons immediately after sugar reward learning cripples behavioral approach measured 24 h later [50•], although a functional connection between the glutamatergic α1 MBON and α1 DANs has not been demonstrated. **(c)** A feedback and feed-across recurrency is crucial for memory reconsolidation. Experiencing training-related cues can switch stable memories back into a labile state and reconsolidation is then required for the memory to return to a persistent condition [9••]. The reconsolidation process requires the activity of the cholinergic MBON-γ2α′1 which in turn recruits two different sets of dopaminergic neurons: the PPL1-γ2α′1 DANs, which are required during memory retrieval, and a group of PAM-DANs which innervate different compartments and are required in the period following retrieval. These cholinergic MBON-DAN connections are excitatory [9••].

comprised of the MP1 DANs [21] and a pair of GABAergic neurons, called MVP2 (or MBON-γ1pedc > αβ), that exert feedforward inhibition across the MBON network [12••] (Figure 3a). In hungry flies, inhibition from MVP2

Internal states and types of learning

- Well fed. - Think about something other than food, probably mating.
- Courting. - Approach conspecifics. Try to mate.
- Mated. - Mated females become much more interested in food and reject further courtship. Males learn not to court mated females. This is Courtship conditioning training.



Review

Diverse memory paradigms in *Drosophila* reveal diverse neural mechanisms

Amoolya Sai Dwijesha,[1,4] Akhila Eswaran,[1,4] Jacob A. Berry,[1,2] and Anna Phan[1,2,3]

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In this review, we aggregated the different types of learning and memory paradigms developed in adult *Drosophila* and attempted to assess the similarities and differences in the neural mechanisms supporting diverse types of memory. The simplest association memory assays are conditioning paradigms (olfactory, visual, and gustatory). A great deal of work has been done on these memories, revealing hundreds of genes and neural circuits supporting this memory. Variations of conditioning assays (reversal learning, trace conditioning, latent inhibition, and extinction) also reveal interesting memory mechanisms, whereas mechanisms supporting spatial memory (thermal maze, orientation memory, and heat box) and the conditioned suppression of innate behaviors (phototaxis, negative geotaxis, anemotaxis, and locomotion) remain largely unexplored. In recent years, there has been an increased interest in multisensory and multicomponent memories (context-dependent and cross-modal memory) and higher-order memory (sensory preconditioning and second-order conditioning). Some of this work has revealed how the intricate mushroom body (MB) neural circuitry can support more complex memories. Finally, the most complex memories are arguably those involving social memory: courtship conditioning and social learning (mate-copying and egg-laying behaviors). Currently, very little is known about the mechanisms supporting social memories. Overall, the MBs are important for association memories of multiple sensory modalities and multisensory integration, whereas the central complex is important for place, orientation, and navigation memories. Interestingly, several different types of memory appear to use similar or variants of the olfactory conditioning neural circuitry, which are repurposed in different ways.

Figure 2. Courtship and social learning paradigms. (*A*) In courtship conditioning paradigms, male flies are conditioned to have their courtship advances rejected by a mated or immature female, reducing their subsequent courtship attempts with other females. (*B*) During mate-copying, an observer female will prefer to mate with a green-colored male (panel *i*) or with an unhealthy male if they previously observed a demonstrator female mating with them (panel *ii*). (*C*) Social learning of egg-laying preference information is transmitted from the demonstrator to the observer to lay eggs at sites preferred by other mated demonstrator females (panel *i*) or to reduce egg laying due to the presence of parasitoid wasps (panel *ii*).

Internal states and types of learning

- Sociable. - Flies congregate at food sources.
- Females release a pheromone that attracts both males and females. Also, they place this pheromone at egg-laying sites to get more females to lay eggs there. Larvae help each other to soften food.

- Imitating others. Surprisingly, flies also learn by watching others.

- A demonstrator female mates with a male fly dusted with one color and then rejects a male dusted with another color. The Observer female is likely to copy her.
- A demonstrator female lays eggs in one spot and rejects another spot. Observer will likely copy.
- A demonstrator female reduces egg laying in the presence of parasitic wasps that lay eggs in larvae. Observer female does the same. May involve some visual communication from the demonstrator female to the observer using the wings.

Innate immunity and sleep.

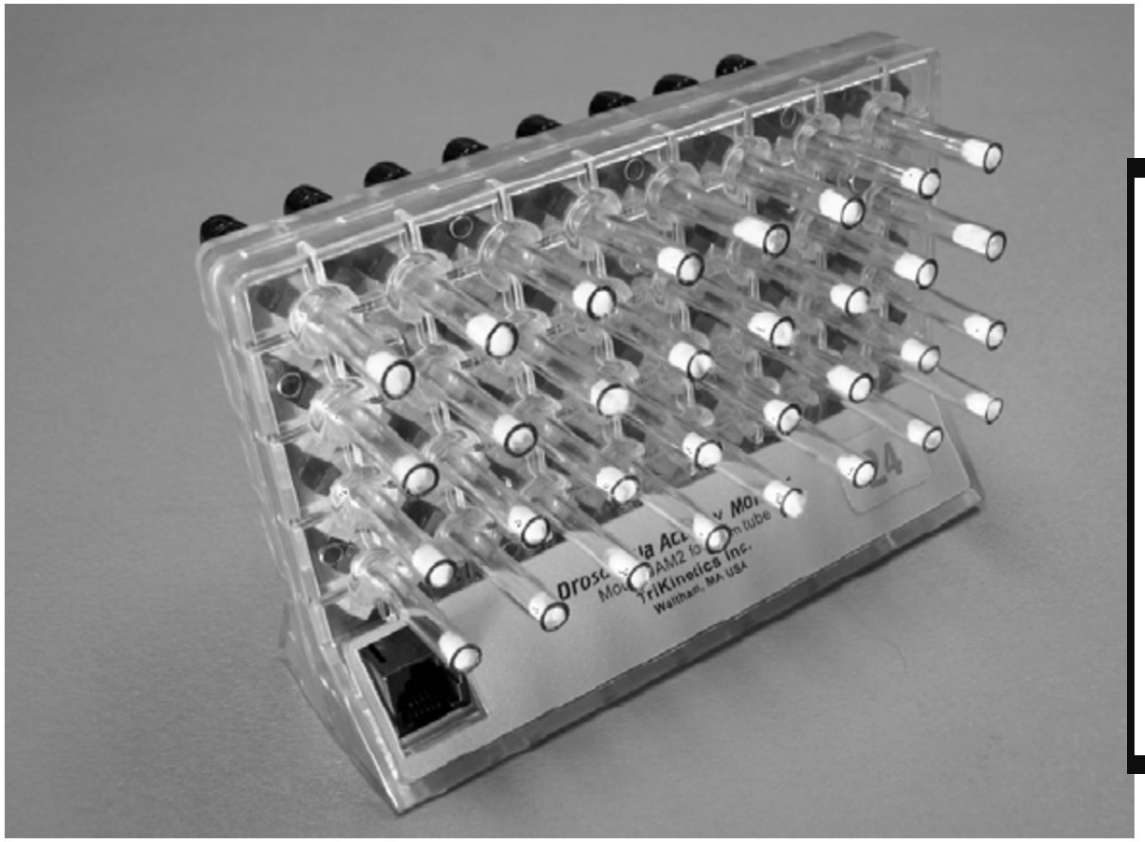
- Flies learn to avoid food that gives them infections.

- Sleep is another of the many possible behavioral choices dictated by internal states; sleep need in this case.
- Severe hunger suppresses the need for sleep without the buildup of sleep deprivation.
- Sleeping flies have reduced responsiveness but wake for food odors if hungry.

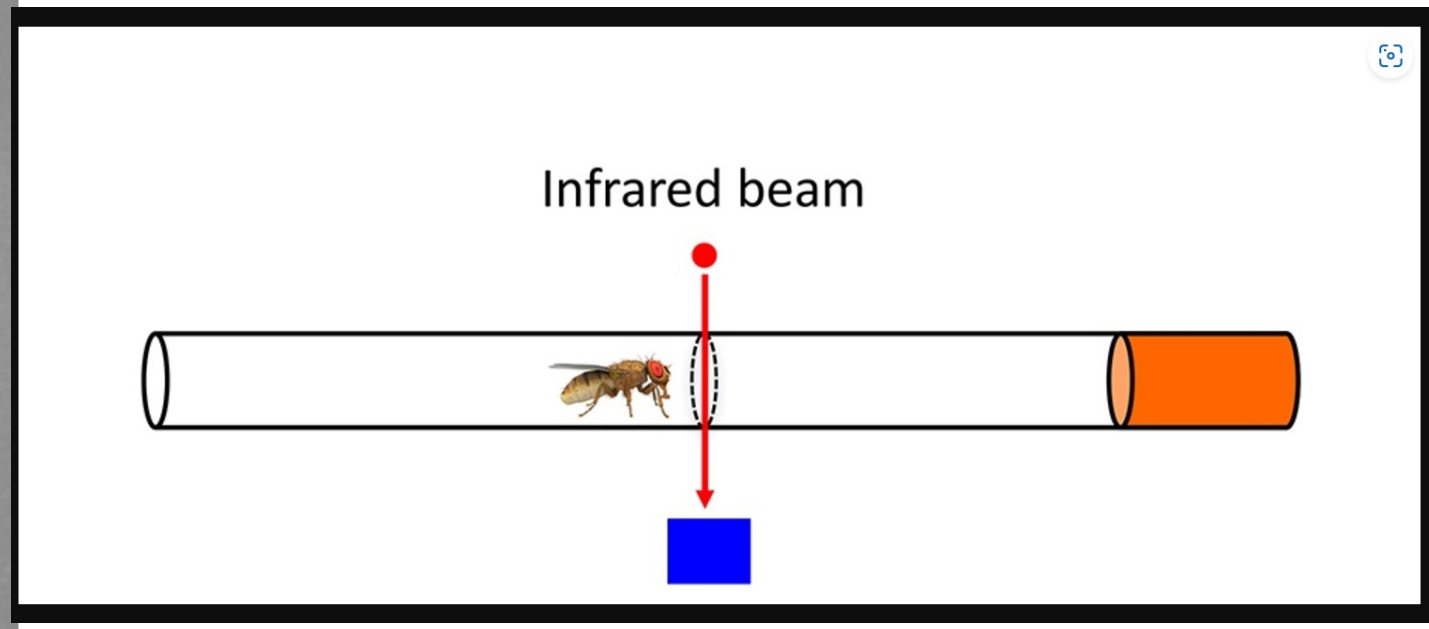
Circadian rhythms

Drosophila Activity Monitor (DAM).

One fly per tube. 32 tubes. Tubes have food, for days to weeks.



Drosophila activity monitor.



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Clock Mutants of *Drosophila melanogaster*

(eclosion/circadian/rhythms/X chromosome)

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Contributed by Seymour Benzer, July 2, 1971

ABSTRACT **Three mutants have been isolated in which the normal 24-hour rhythm is drastically changed. One mutant is arrhythmic; another has a period of 19 hr; a third has a period of 28 hr. Both the eclosion rhythm of a population and the locomotor activity of individual flies are affected. All these mutations appear to involve the same functional gene on the X chromosome.**

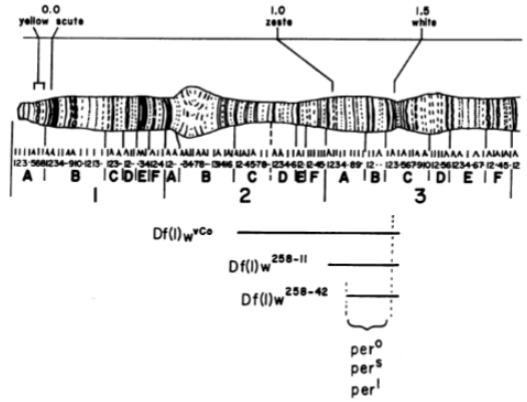

Fig. 4. Bridges' map (28) of the X chromosome of *Drosophila melanogaster*, showing about one-sixth of the chromosome at the end distal to the centromere. Each deficiency mutant lacks bands over the range indicated (9). per^0 = arrhythmic mutant, per^s = short-period mutant, per^l = long-period mutant.

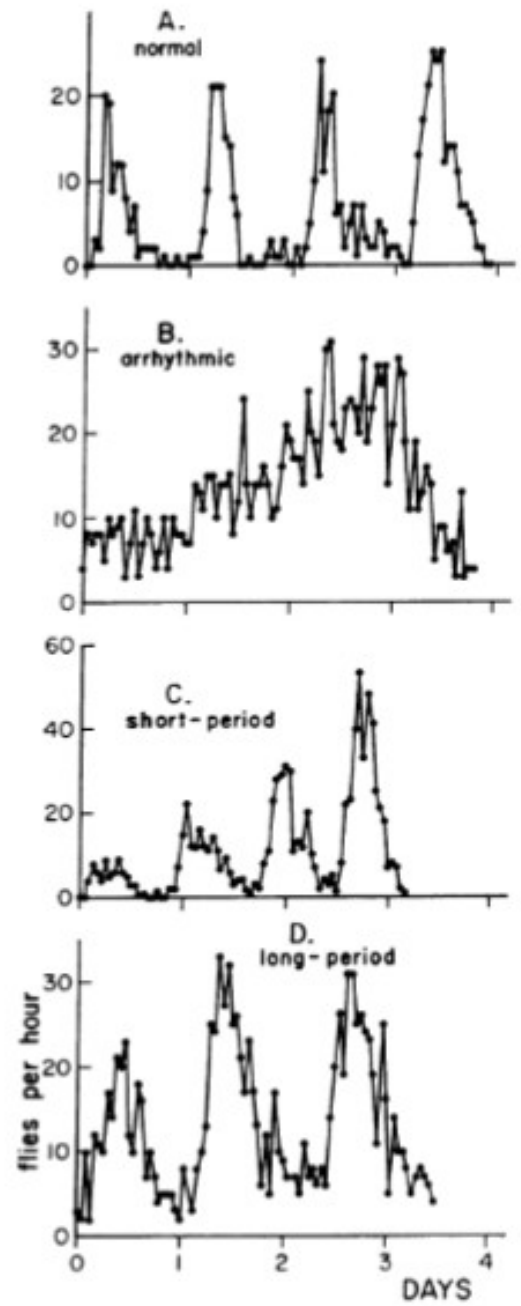

FIG. 1. Eclosion rhythms, in constant darkness, for populations of rhythmically normal and mutant flies, previously exposed to LD 12:12. T = 20°C.

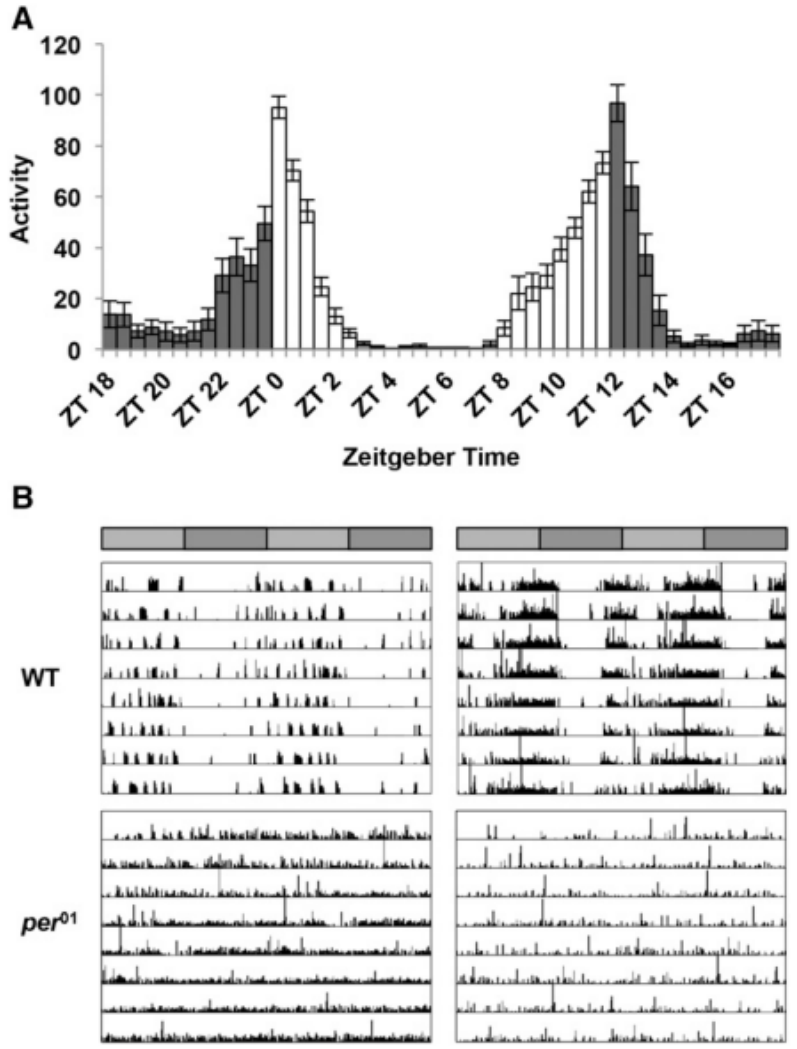

Figure 1 (A) Activity for a group of wild-type (WT) male flies in a 12:12 hr light:dark (LD) cycle at 25°. Flies anticipate lights-off, and under these conditions also lights-on, with increased activity in advance of these transitions. (B) Double-plotted activity in constant darkness for two individual WT and *per*^{01} male flies after entrainment in standard LD conditions. Data are double-plotted for ease of interpretation, with each day of data plotted in a new row concatenated with the data from the subsequent day, such that Row 1 displays data for Day 1 and Day 2, Row 2 displays data for Day 2 and Day 3, etc. Activity is concentrated in the subjective day in a pattern that recurs with a period of slightly <24 hr in WT flies.

Circadian Rhythms and Sleep in *Drosophila melanogaster*

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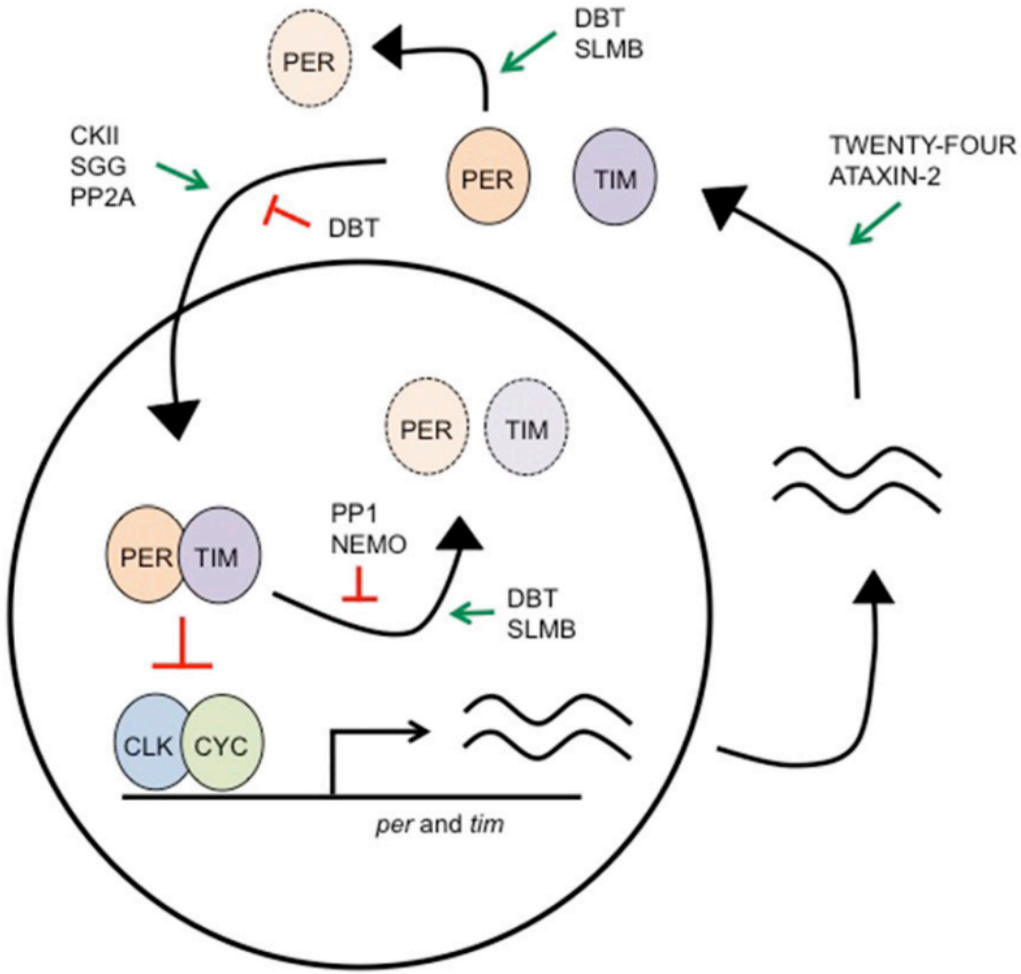

Figure 2 The molecular feedback loop is formed by the negative feedback of Period (PER) and Timeless (TIM) on their own transcription. Delays exist between transcription of *per* and *tim* mRNA and the localization of these proteins in the nucleus, where they can interact with transcriptional activators Clock (CLK) and Cycle (CYC). These delays are thought to be important for allowing the molecular clock to cycle with a period of ~24 hr. Critical regulators have been identified at several steps of the cycle that are necessary for accurate timing and strength of molecular rhythms. Degradation of PER and TIM allows the cycle to start anew. Not pictured is the second feedback loop formed by PDP1 and Vrille, which produces cycling of *Clk* mRNA. This secondary feedback loop is thought to reinforce molecular oscillations, although cycling of the CLK protein is not necessary for rhythms. CKII, Casein Kinase II; SGG, shaggy; PP2A, Protein Phosphatase 2A; PP1, Protein Phosphatase 1; DBT, doubletime.

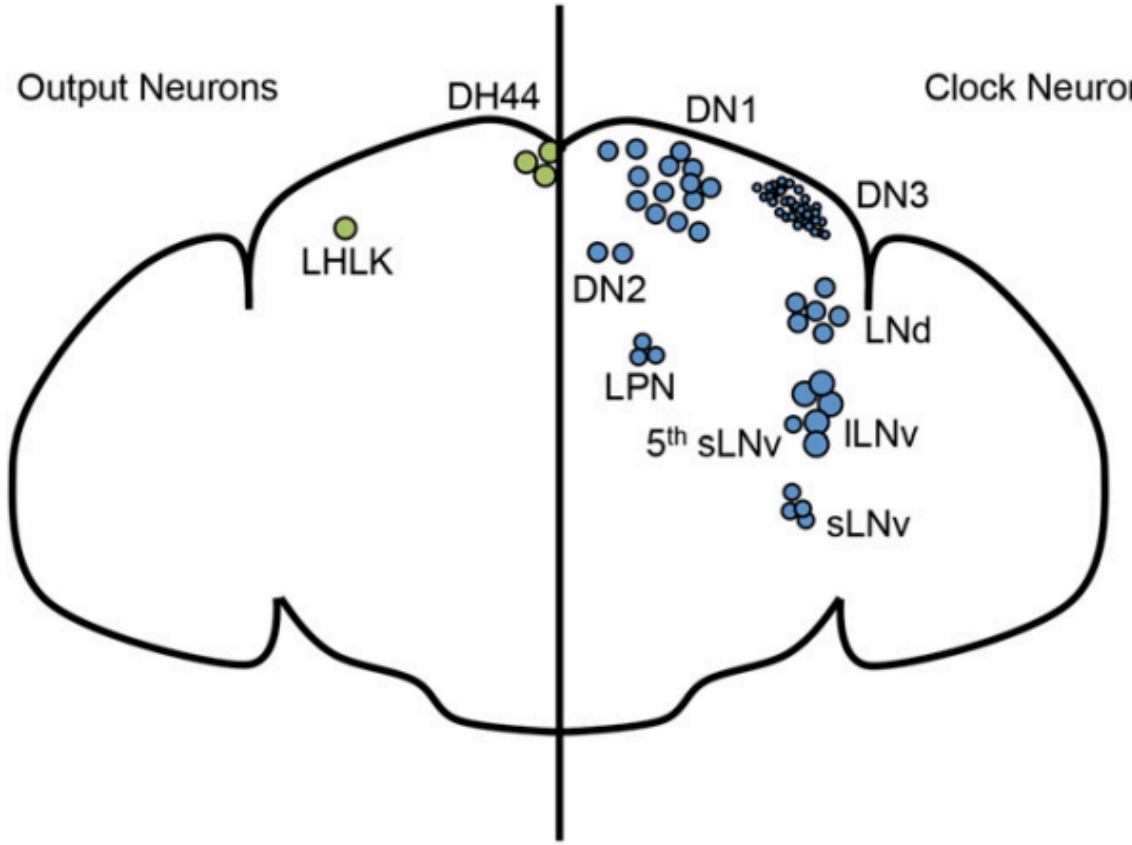

Figure 3 Clock cells, which express the core components of the molecular clock, are depicted on the right. These cells are interconnected and heterogeneous between and within clusters, allowing cells to serve different functions, and respond to different environmental conditions. On the left, two groups of output neurons that do not express the molecular components of the clock, but have cycling neuronal activity and are important for behavioral activity rhythms, suggesting clock input. DN, dorsal neurons; LN, lateral neurons (lLNv, large ventral lateral neurons; sLNv, small ventral lateral neurons; LNd, dorsal lateral neurons); LPN, lateral posterior neurons; LHLK, lateral horn leucokinin neuron; DH44, Diuretic Hormone 44.

bioRχiv

Synaptic connectome of the *Drosophila* circadian clock

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Table 1: Identification and classification of *Drosophila* clock neurons in the FlyWire brain connectome

Clock Super Class	Clock Class	Clock Cell Type	Expected	Observed
Dorsal neurons (DN)	anterior DN_1 (DN_{1a})	DN_{1a}	4	4
	posterior DN_1 (DN_{1p})	$DN_{1p}A$	~8-10	8
		$DN_{1p}B$	~4	4
		$DN_{1p}C$	~12-14	4
		$DN_{1p}D$		8
		$DN_{1p}E$		4
	DN_2	DN_2	4	3
	DN_3	small Central Projecting DN_3 (s-$CPDN_3$)A	~80	38
		s-$CPDN_3$B		25
		s-$CPDN_3$C		32
		s-$CPDN_3$D		37
		s-$CPDN_3$E		25
		large Central Projecting DN_3 (l-$CPDN_3$)		2
		Anterior Projecting DN_3 ($APDN_3$)		12
Lateral neurons (LN)	Lateral Posterior Neurons (LPN)	LPN	6	6
	dorsoLateral Neurons (LN_d)	Cryptochrome-negative LN_d (LN_d^{CRY-})	6	6
		Cryptochrome-positive LN_d (LN_d^{CRY+})	4	4
	ITP-positive Lateral Neurons (LN^{ITP})	Cryptochrome- and ITP-positive LN_d ($LN_d^{CRY+ \& ITP}$)	2	2
		5th ventroLateral Neuron (5th-LN_v)	2	2
	PDF-positive ventroLateral Neurons (LN_v^{PDF})	large ventroLateral Neuron (l-LN_v)	8	8
		small ventroLateral Neuron (s-LN_v)	8	8
		Total	**~152**	**242**

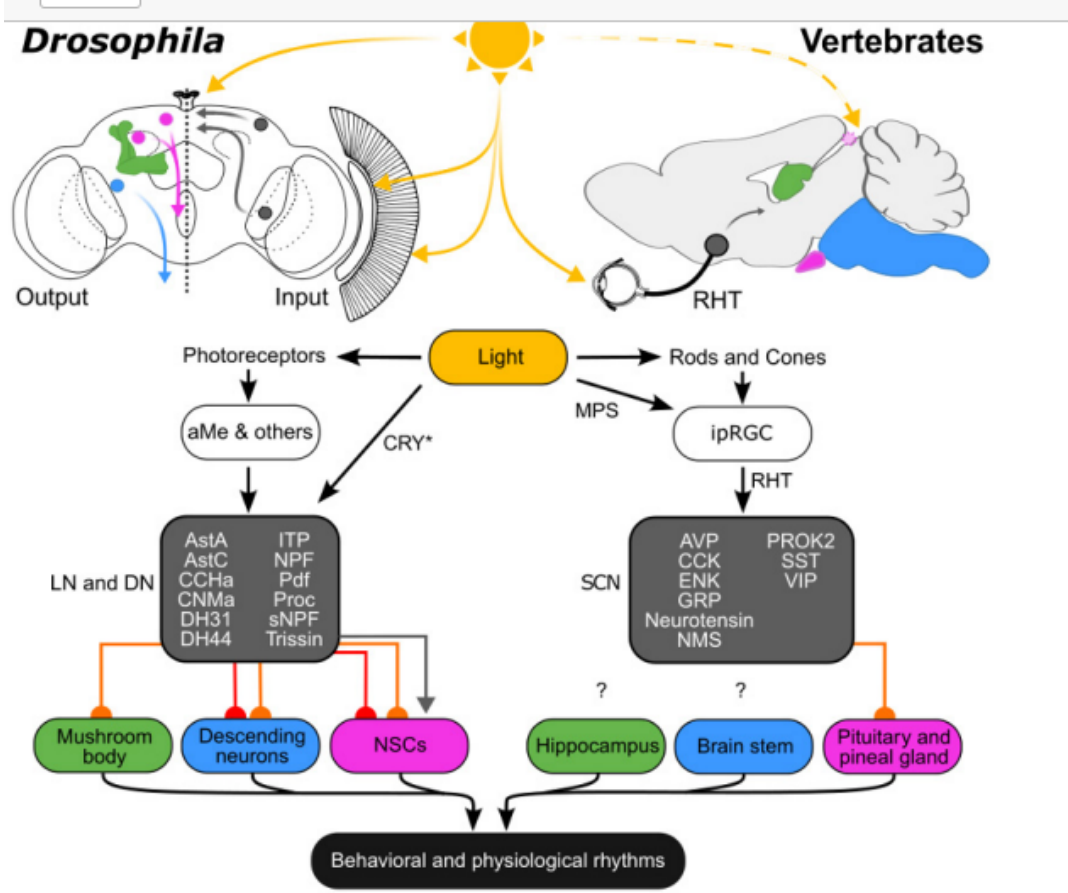

Figure 8: Parallels in *Drosophila* and vertebrate clock input and output pathways. Direct and indirect light input pathways to the *Drosophila* clock (comprised of LN and DN) and vertebrate suprachiasmatic nucleus (SCN). Note that the pineal gland receives light input only in non-mammalian vertebrates. *Drosophila* and vertebrate clocks utilize different neuropeptides (grey box). Output from the clock to downstream targets is either synaptic (direct in red and indirect in orange) or paracrine (grey arrow). Abbreviations: CRY, Cryptochrome; MPS, Melanopsin, RHT, Retinohypothalamic tract; ipRGC, Intrinsically-photosensitive retinal ganglion cells; aMe, Accessory medulla neurons; NSC, neurosecretory cell; AVP, arginine vasopressin; CCK, cholecystokinin; ENK, met-enkephalin; GRP, gastrin-releasing peptide; NMS, neuromedin S; PROK2, prokineticin 2; SST, somatostatin; VIP, vasoactive intestinal peptide.

What is sleep for?

Sleep is needed for learning and memory. *Rutabaga* and *dunce* learning defects are suppressed by enforced sleep,

A related idea is that sleep is needed for synaptic homeostasis to maintain neuronal plasticity for learning.

CNS proteins may be made during sleep that enable memrory consolidation.

Table 1 | **Genes reported, or suspected, to be involved in *Drosophila melanogaster* memory**

Mutant/transgene (gene)	Abbreviation	Product	Expression	Refs
amnesiac	*amn*	Putative neuropeptide	High in DPM	149
dunce	*dnc*	cAMP phosphodiesterase	High in MBs	123
rutabaga	*rut*	Type I adenylyl cyclase	High in MBs	128
turnip	*tur*	ND	ND	165
radish	*rsh*	No obvious functional homologue	High in MBs	29
DC0	*DC0*	PKA catalytic subunit	High in MBs	132
PKA-RI	*PKA-RI*	PKA regulatory subunit	High in MBs	166
G-sα60A	*G-sα60A*	Stimulatory G protein	ND	137
Neurofibromin	*NF1*	Ras-GTPase activating protein (GAP)	ND	167
leonardo	*leo*	14-3-3ζ	High in MBs*	168
volado (scab)	*scb*	α-integrin	High in MBs*	138
fasciclin II	*fasII*	Neural cell adhesion molecule	High in MBs*	139
damb	*damb*	Dopamine receptor in MBs	High in MBs	119
dDA1/DMDOP1	*dDA1*	Dopamine receptor	High in MBs	120
oamb	*oamb*	Octopamine receptor in MBs	High in MBs	121
mushroom body miniature	*mbm*	Transcription factor	High in MBs‡	122
mushroom bodies deranged	*mbd*	ND	ND	122
ignorant (S6KII)	*S6KII*	Ribosomal S6 kinase	ND	169
latheo	*lat*	Origin recognition complex	ND	170
nalyot (Adf1)	*Adf1*	ADF1 transcription factor	Widespread	171
linotte (derailed)	*drl*	Receptor tyrosine kinase	High in MBs	172
milord (pumilio)	*pum*	RNA binding protein/ translation	High in MBs§	140
norka (oskar)	*osk*	Translation control	High in MBs§	140
krasavietz (eIF-5C)	*eIF-5C*	Translation initiation factor	High in MBs§	140
staufen	*stau*	RNA binding protein/ translocation	ND	140
armitage	*armi*	RNA-induced silencing complex	ND	70
Nmdar1	*Nmdar1*	Glutamate receptor subunit	Widespread	173
Nmdar2	*Nmdar2*	Glutamate receptor subunit	Widespread	173
dCREB2 (CrebB-17A)	*CrebB-17A*	cAMP-responsive transcription factor	Widespread	174
nebula (sarah)	*sra*	Calcineurin inhibitor	ND	175
Tbh	*Tbh*	Tyramine β-hydroxylase	Octopaminergic neurons	26
Notch	*N*	Cell surface receptor	ND	176,177
crammer	*cre*	Cysteine protease inhibitor	MBs and glia	178
aPKC	*aPKC*	Atypical protein kinase C	ND	179
tequila	*teq*	Neurotrypsin	MBs‖	143
synapsin	*syn*	Presynaptic vesicle protein	Widespread	180
55 additional Pavlov's/Tully's dogs¶		Various	Many in MBs§	140

Sleep

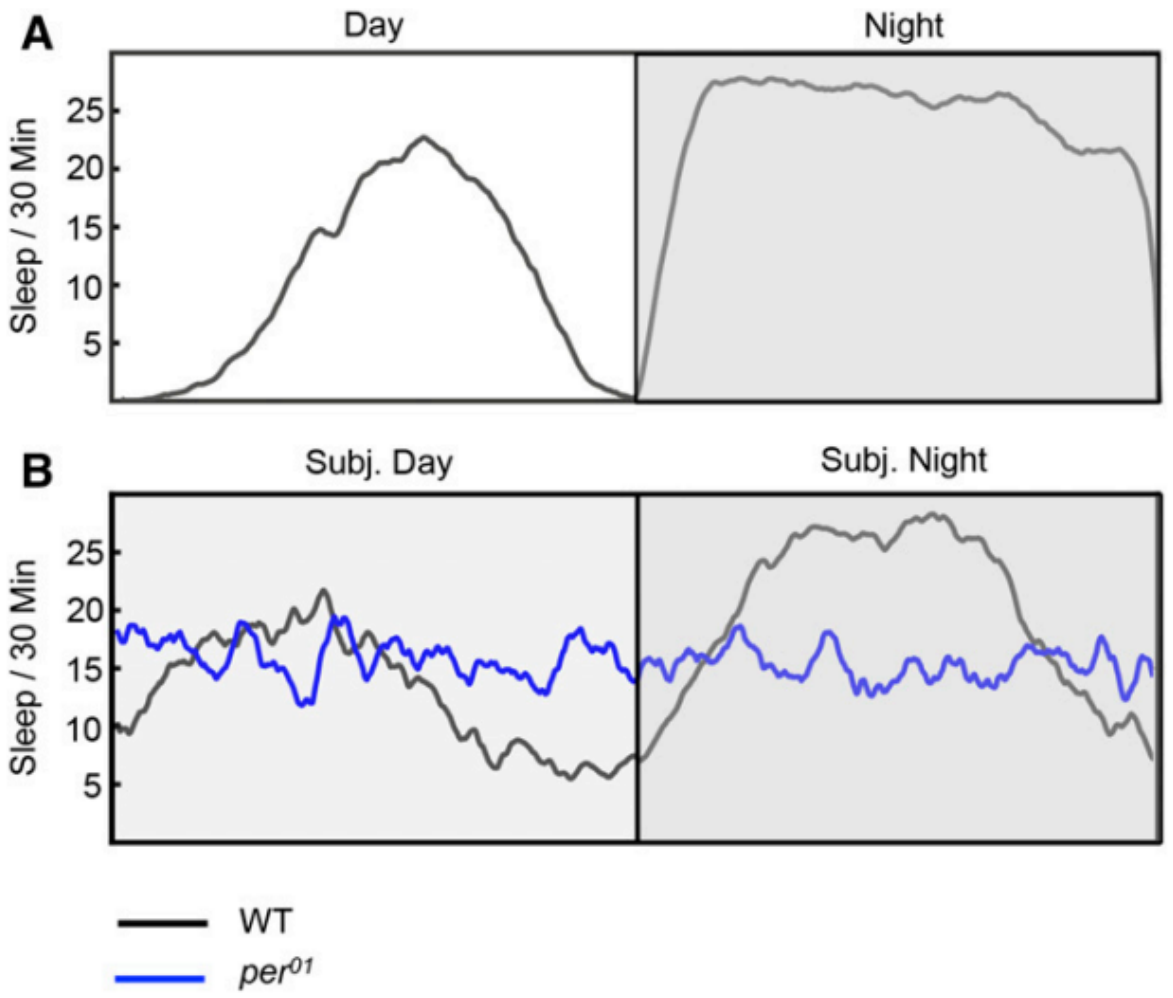

Figure 4 (A) Sleep behavior for a group of wild-type (WT) female flies in a 12:12 hr light:dark cycle. Flies have short bouts of siesta sleep in the middle of the day (more pronounced in males) and a relatively consolidated period of sleep at night. (B) Sleep behavior for WT and *per*^{01} male flies in constant darkness (DD). *per*^{01} flies, which do not display circadian rhythms of activity, spend approximately the same amount of time in sleep, but have sleep that is fragmented across the day. Data appear slightly noisier as fewer flies are represented compared to (A).

THEORETICAL REVIEW

Sleep function and synaptic homeostasis

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KEYWORDS
Long-term depression;
Synaptic scaling;
Learning;
Consolidation;
Delta sleep;
Slow waves;
Slow oscillation

Summary This paper reviews a novel hypothesis about the functions of slow wave sleep—the synaptic homeostasis hypothesis. According to the hypothesis, plastic processes occurring during wakefulness result in a net increase in synaptic strength in many brain circuits. The role of sleep is to downscale synaptic strength to a baseline level that is energetically sustainable, makes efficient use of gray matter space, and is beneficial for learning and memory. Thus, sleep is the price we have to pay for plasticity, and its goal is the homeostatic regulation of the total synaptic weight impinging on neurons. The hypothesis accounts for a large number of experimental facts, makes several specific predictions, and has implications for both sleep and mood disorders.

Widespread Changes in Synaptic Markers as a Function of Sleep and Wakefulness in *Drosophila*

Giorgio F. Gilestro, Giulio Tononi,* Chiara Cirelli*

Sleep is universal, strictly regulated, and necessary for cognition. Why this is so remains a mystery, although recent work suggests that sleep, memory, and plasticity are linked. However, little is known about how wakefulness and sleep affect synapses. Using Western blots and confocal microscopy in *Drosophila*, we found that protein levels of key components of central synapses were high after waking and low after sleep. These changes were related to behavioral state rather than time of day and occurred in all major areas of the *Drosophila* brain. The decrease of synaptic markers during sleep was progressive, and sleep was necessary for their decline. Thus, sleep may be involved in maintaining synaptic homeostasis altered by waking activities.

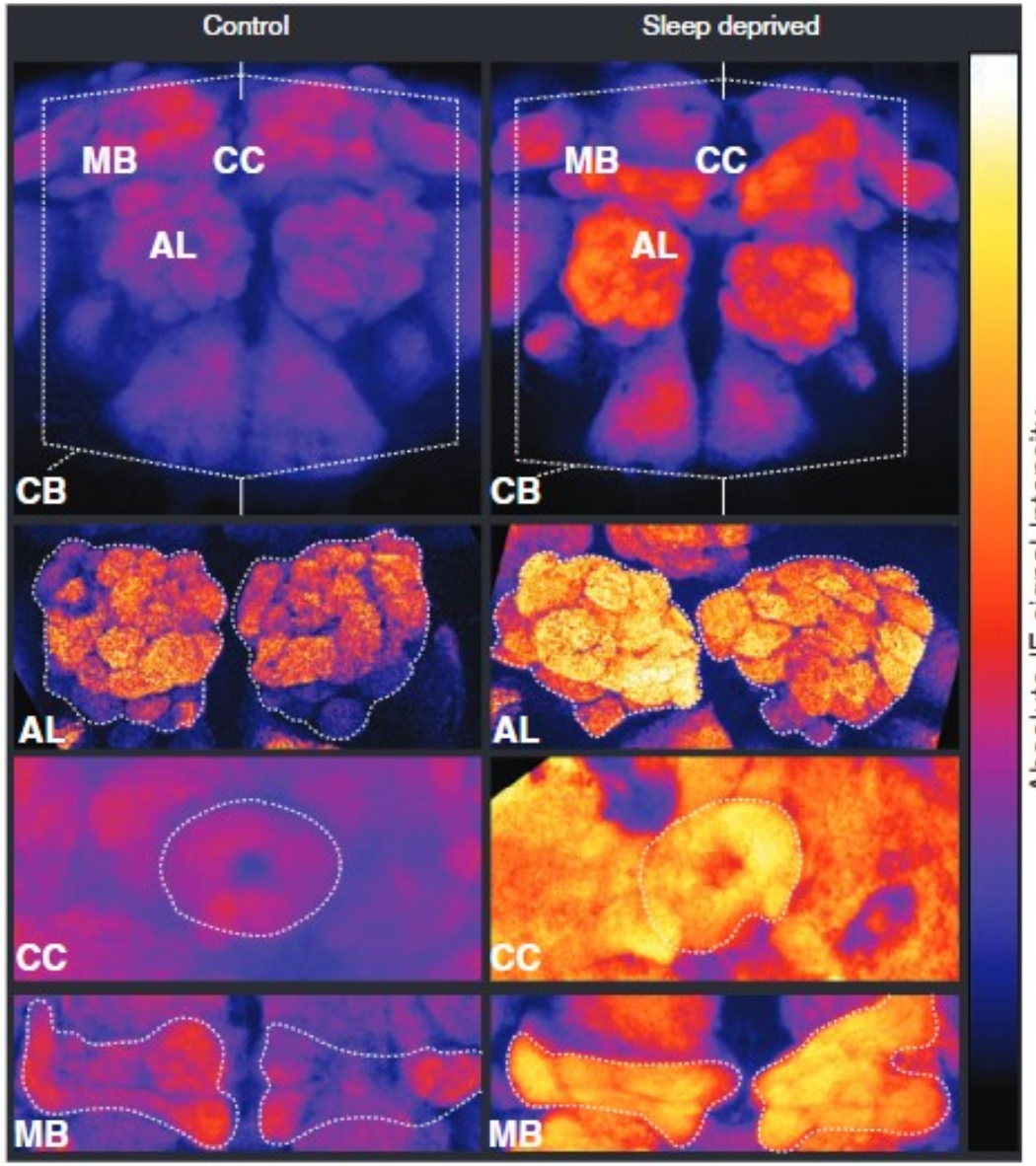

Fig. 4. Widespread BRP increase after sleep loss, as shown by representative examples of BRP immunofluorescence (IF) in controls and flies sleep-deprived for 16 hours (sleep loss >80%) ending at light onset (sum of selected optical stacks, false-colored on a quantitative scale). Immunoreactivity levels were measured in antennal lobes (AL), β lobes of the mushroom bodies (MB), ellipsoid body of the central complex (CC), and central cerebrum excluding the optic lobes (CB).

RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

The forebrain synaptic transcriptome is organized by clocks but its proteome is driven by sleep

Sara B. Noya, David Colameo, Franziska Brüning, Andrea Spinnler, Dennis Mircsof, Lennart Opitz, Matthias Mann, Shiva K. Tyagarajan*, Maria S. Robles

INTRODUCTION: Temporally consolidated behaviors such as sleep normally occur in synchrony with endogenous circadian rhythms and both have been reported to contribute to global daily oscillations of transcription in brain. Neurons have further adapted specialized means to traffic RNA into distant dendritic and axonal arbors, where it is locally translated. Together, these mechanisms allow coordination of physiology with environmental needs.

RATIONALE: About 6% of the forebrain transcriptome oscillates in a time-of-day–dependent manner, and it has been proposed that this oscillation is mostly driven by the sleep-wake state to enable daily changes in synaptic structure and function. In turn, such synaptic scaling is thought to form a critical feature of the sleep-

of this temporal gating for synaptic function and energy homeostasis. Overall, the oscillations of 75% of synaptic proteins were concomitant with their rhythmic transcripts, indicating a key role for local synaptic translation. Under conditions of high sleep pressure, one-fourth of mRNAs remained identically circadian, and most preserved some degree of circadian

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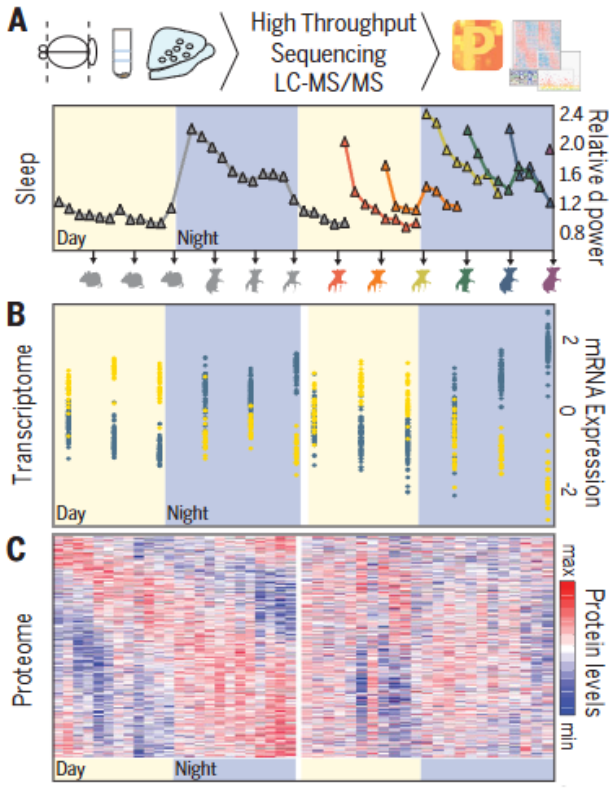
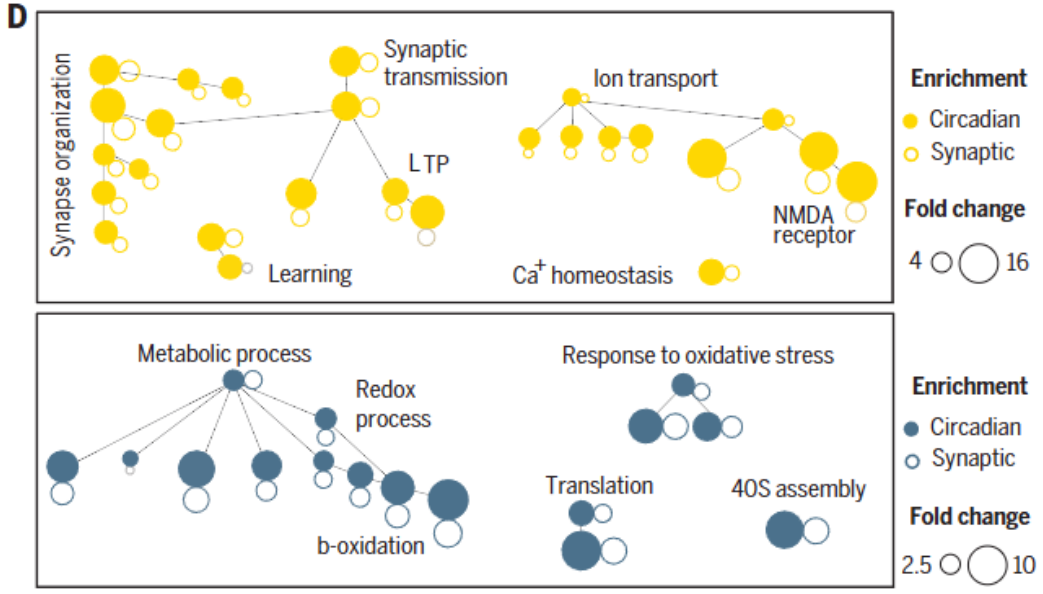




Circadian clocks regulate synaptic mRNAs but sleep and wake regulate their proteins. (**A**) Workflow: Forebrain synaptoneurosomes were isolated across the day at low and high sleep pressure. (**B**) Synaptic transcripts can maintain circadian rhythmicity under high sleep pressure (**C**) but protein rhythms are completely abolished. (**D**) Gene ontology highlights the complete temporal segregation of predusk (top) and predawn (bottom) synaptic function.

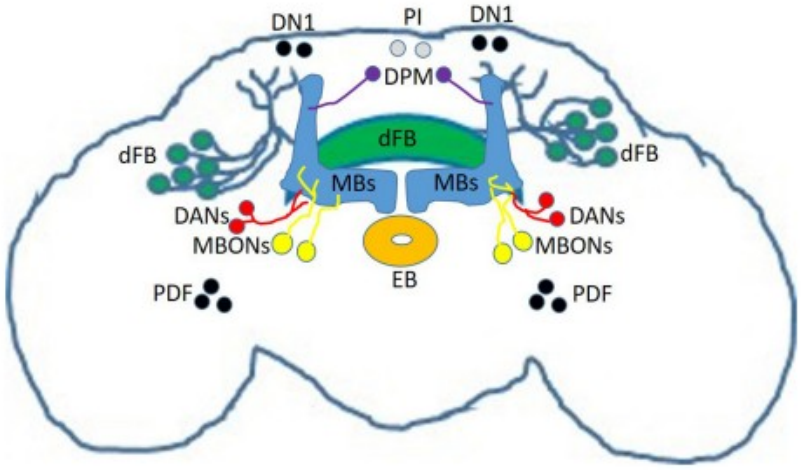
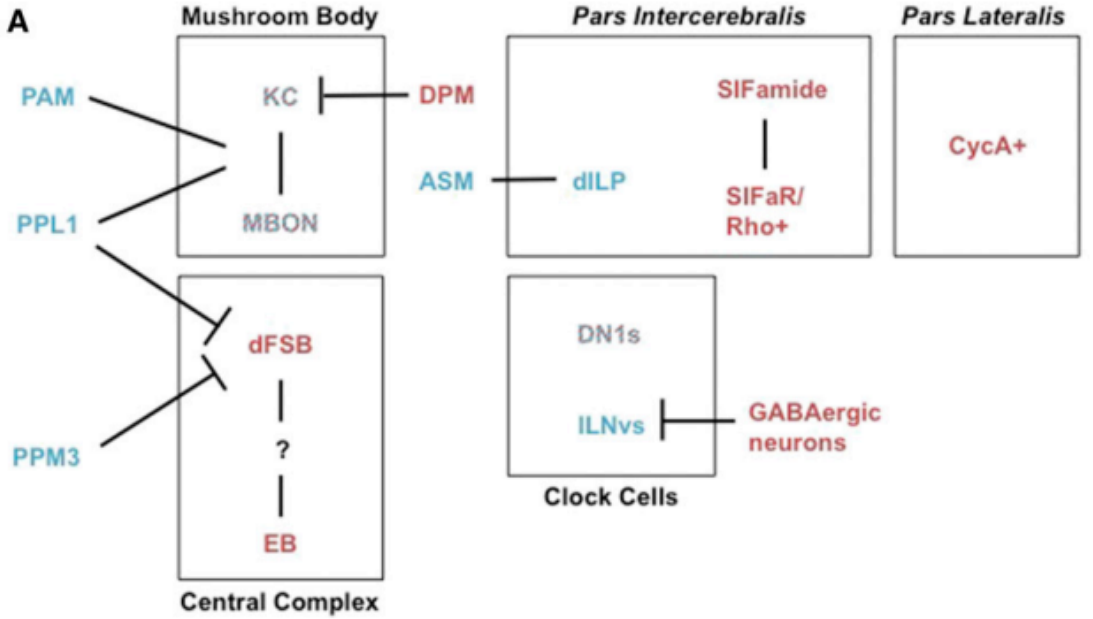


FIGURE 1 | Sleep-regulating circuits in the fly brain. Schematic representation of neurons and circuits involved in sleep regulation including Mushroom Bodies (MBs, blue); Mushroom Body Output Neurons (MBONs, yellow); dopaminergic neurons (DANs, red); circadian clock neurons (black) including Pigment-Dispersing Factor (PDF) neurons and Dorsal Neurons 1 (DN1); Ellipsoid Body neurons (EB, orange); dorsal Fan-Shaped Body neurons (dFB, green); Pars Intercerebralis neurons (PI, gray) and Dorsal-Paired Medial Neurons (DPM, purple).



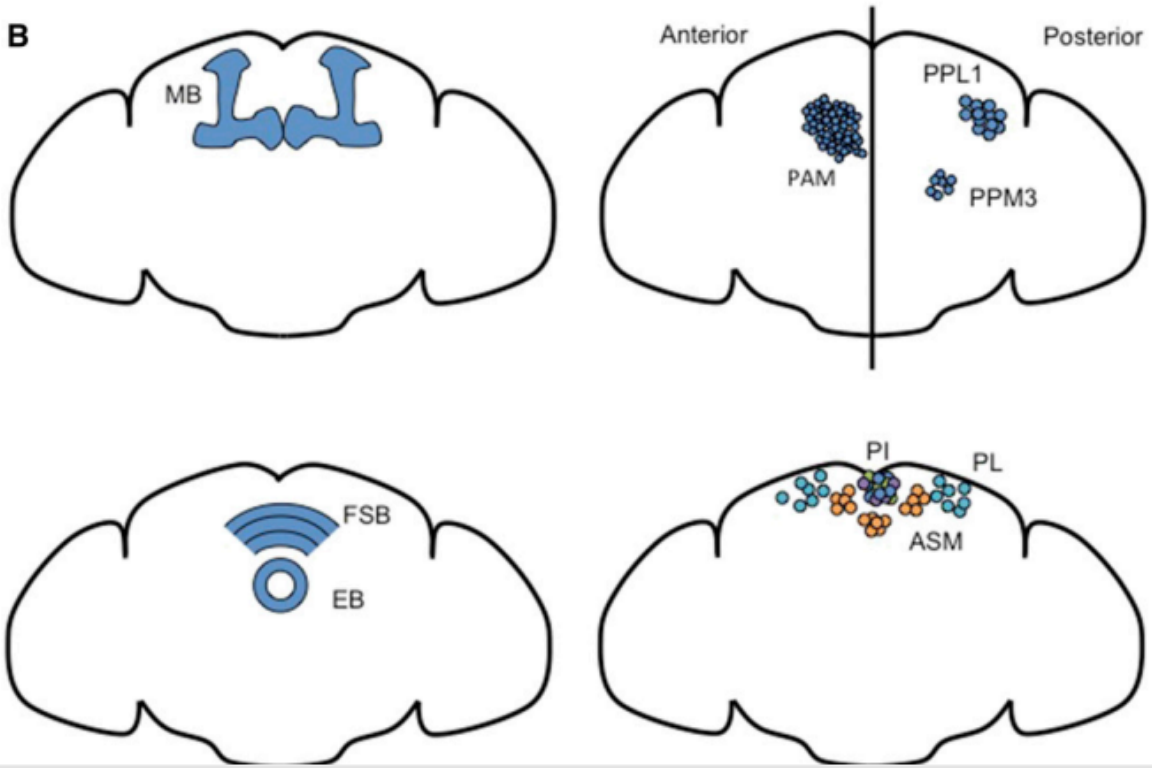


Figure 5 (A) Schematic of sleep-promoting (red), and sleep-inhibiting (blue), neurons in the fly brain. Sleep-regulating neurons are identified by neurotransmitter, neuropeptide, or molecular marker expression, and/or neuroanatomic location. Dopaminergic neurons: PAM, protocerebral anterior lateral; PPL1, protocerebral posterior lateral; and PPM3, protocerebral posterior medial. Mushroom body (MB) neurons: KC, Kenyon cells; MBON, mushroom body output neurons. Central complex: dFSB, dorsal fan-shaped body; EB, ellipsoid body. *Pars intercerebralis* (PI): SIFaR, SIFamide Receptor; Rho, Rhomboid; and dILP, *Drosophila* insulin-like peptide. Octopaminergic neurons: ASM, anterior superior medial. *Pars lateralis* (PL): CycA, CyclinA. Clock cells: DN, dorsal neurons; lLNvs, large ventral lateral neurons. (B) Location of sleep-regulating neurons in the fly brain.

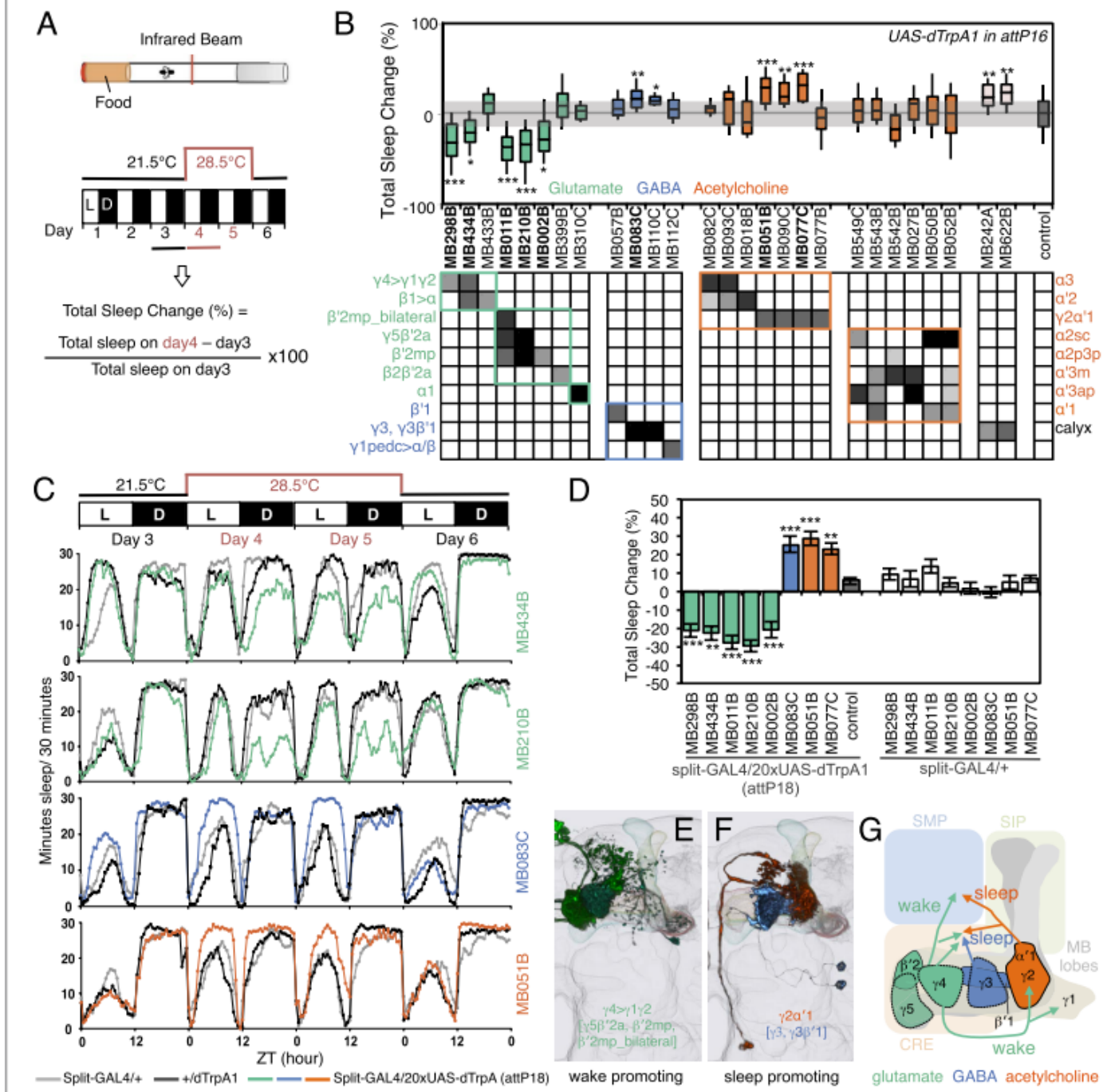
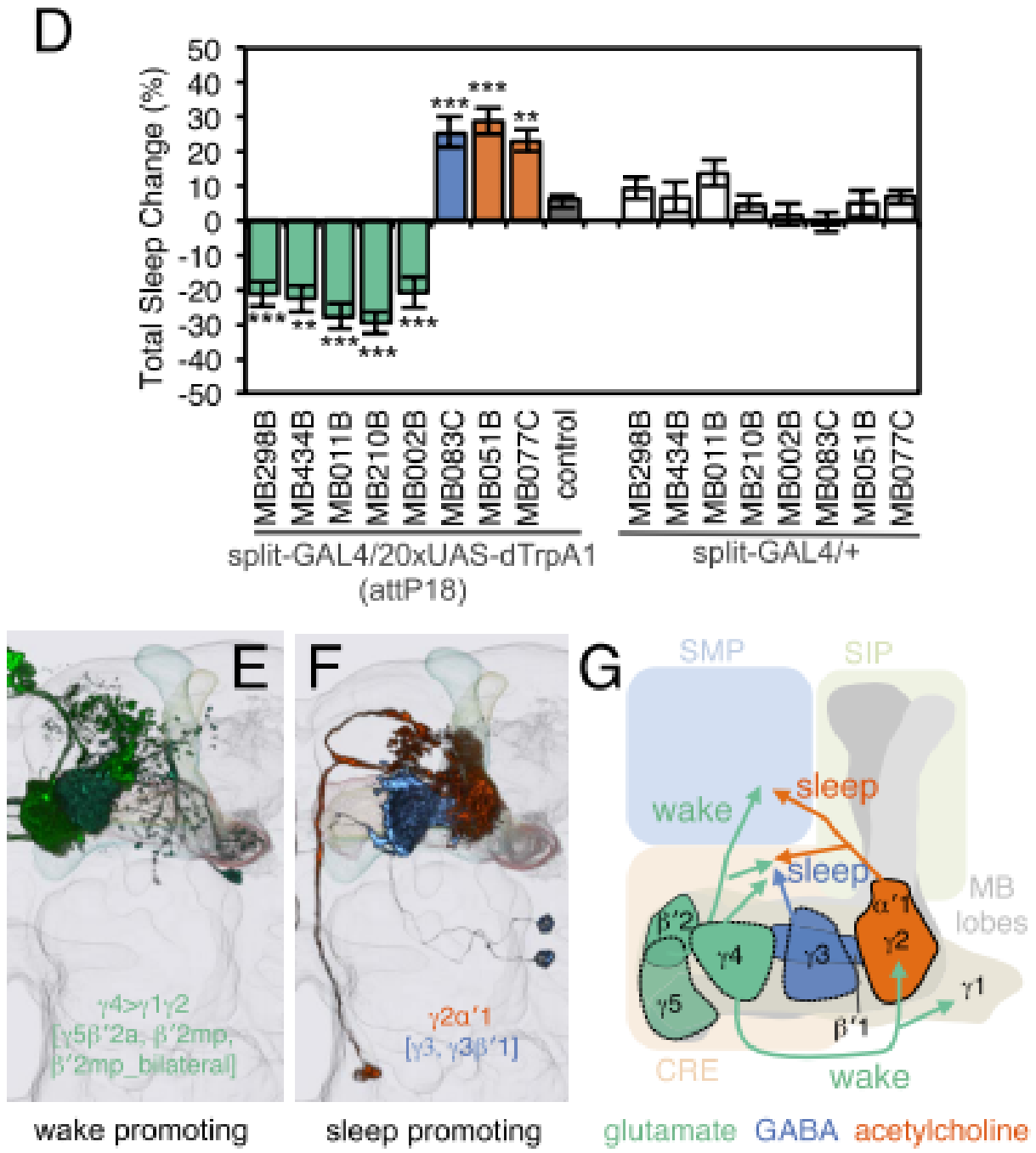

A
Infrared Beam
Food
21.5°C
28.5°C
L D
Day 1 2 3 4 5 6
Total Sleep Change (%) =
Total sleep on day4 – day3
Total sleep on day3
x100
B
UAS-dTrpA1 in attP16
Total Sleep Change (%)
100
0
-100
Glutamate GABA Acetylcholine
MB298B MB434B MB433B MB011B MB210B MB002B MB399B MB310C MB057B MB083C MB110C MB112C MB082C MB093C MB018B MB051B MB090C MB077C MB077B MB549C MB543B MB542B MB027B MB050B MB052B MB242A MB622B control
γ4>γ1γ2
β1>α
β'2mp_bilateral
γ5β'2a
β'2mp
β2β'2a
α1
β'1
γ3, γ3β'1
γ1pedc>α/β
α3
α'2
γ2α'1
α2sc
α2p3p
α'3m
α'3ap
α'1
calyx
C
21.5°C
28.5°C
L D L D L D L D
Day 3 Day 4 Day 5 Day 6
Minutes sleep/ 30 minutes
MB434B
MB210B
MB083C
MB051B
ZT (hour)
Split-GAL4/+
+/dTrpA1
Split-GAL4/20xUAS-dTrpA (attP18)
D
Total Sleep Change (%)
MB298B MB434B MB011B MB210B MB002B MB083C MB051B MB077C control
split-GAL4/20xUAS-dTrpA1 (attP18)
MB298B MB434B MB011B MB210B MB002B MB083C MB051B MB077C
split-GAL4/+
E
γ4>γ1γ2 [γ5β'2a, β'2mp, β'2mp_bilateral]
wake promoting
F
γ2α'1 [γ3, γ3β'1]
sleep promoting
G
SMP
SIP
wake
sleep
sleep
MB lobes
α'1
β'2
γ2
γ4
γ3
γ5
γ1
β'1
CRE
wake
glutamate GABA acetylcholine

Figure 12. MBONs bi-directionally regulate sleep. (**A**) Top: Schematic of the experimental apparatus used for assaying sleep. Single flies are placed in a tube and activity is measured by counting the number of times the fly crosses an infrared beam. Bottom: Diagram of the experimental assay. Sleep was measured at 21.5°C for 3 days to establish the baseline sleep profile. Flies were then shifted to 28.5°C for 2 days to increase activity of the targeted cells by activating the dTrpA1 channel, and then returned to 21.5°C after activation to assay recovery. The effect of MBON activation on sleep amount is quantified as percentage change in sleep. Negative and positive values indicate decreased and increased sleep, respectively. (**B**) Box plots of change in sleep induced by dTrpA1 [*UAS-dTrpA1* (*attP16*); (***Hamada et al., 2008***)] mediated activation of the neurons targeted by each of the indicated split-GAL4 driver lines. MBONs are grouped by neurotransmitter and color-coded as indicated. The bottom and top of each box represents the first and third quartile, and the horizontal line dividing the box is the median. The whiskers represent the 10^{th} and 90^{th} percentiles. The gray rectangle spanning the horizontal axis indicates the interquartile range of the control. Statistical tests are described in methods: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The matrix below the box plots shows the relative levels of expression (indicated by the gray scale) in the cell types observed with each driver line using *pJFRC200-10XUAS-IVS-myr::smGFP-HA* (*attP18*). Images of the expression patterns of selected drivers are shown in ***Figure 12—figure supplement 1***. (**C**) Sleep profiles of four split-GAL4 lines are shown. Each plot shows a 4-day period starting with subjective dawn. Sleep duration (min/30 min) on day 3 (21.5°C; permissive temperature), days 4, 5 (28.5°C; non-permissive temperature) and day 6 (21.5°C; permissive temperature) are plotted (colored line, split-GAL4 line in combination with *pJFRC124-20XUAS-IVS-dTrpA1* (*attP18*); black line, represents +/dTrpA1; gray line, split-GAL4/+). (**D**) Sleep phenotypes were replicated with *pJFRC124-20XUAS-IVS-dTrpA1* (*attP18*) for the eight drivers shown, but not for MB242A (not shown). Corresponding split-GAL4/+ flies showed normal sleep. (**E**, **F**) Renderings of MBONs responsible for the decreasing (**E**) or increasing (**F**) sleep. MBONs are color-coded based on their putative transmitter. (**G**) Diagram of MBONs responsible for the sleep regulation. MBONs are color-coded based on their putative transmitter as indicated. The wake promoting glutamatergic MBON-γ5β′2a, MBON-β′2mp and MBON-β′2mp_bilateral converge with the sleep promoting cholinergic MBON-γ2α′1 and GABAergic MBON-γ3 and MBON-γ3β′1 in the SMP and CRE. The wake-promoting glutamatergic MBON-γ4>γ1γ2 terminates in the dendritic region of MBON-γ2α′1.

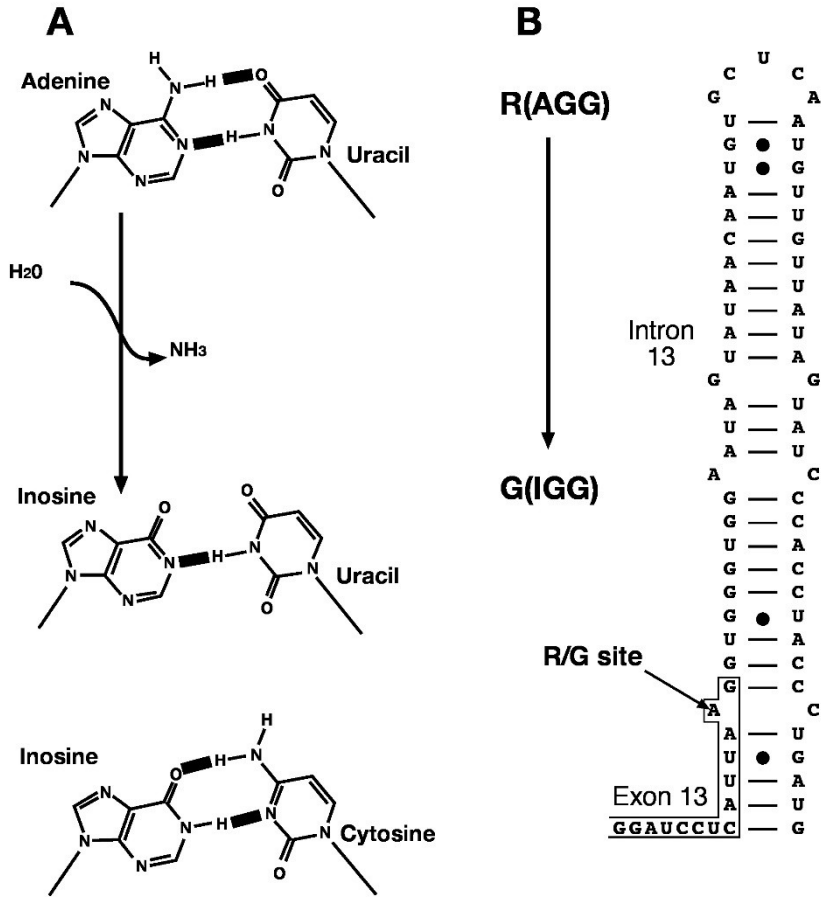

D
Total Sleep Change (%)
50
40
30
20
10
0
-10
-20
-30
-40
-50
***
***
**
***
***
***
**
MB298B
MB434B
MB011B
MB210B
MB002B
MB083C
MB051B
MB077C
control
MB298B
MB434B
MB011B
MB210B
MB002B
MB083C
MB051B
MB077C
split-GAL4/20xUAS-dTrpA1
(attP18)
split-GAL4/+
E
γ4>γ1γ2
[γ5β′2a, β′2mp,
β′2mp_bilateral]
wake promoting
F
γ2α′1
[γ3, γ3β′1]
sleep promoting
G
SMP
SIP
wake
sleep
sleep
MB
lobes
α′1
β′2
γ2
γ4
γ3
γ5
γ1
β′1
CRE
wake
glutamate GABA acetylcholine

ADAR RNA editing enzymes in innate immunity

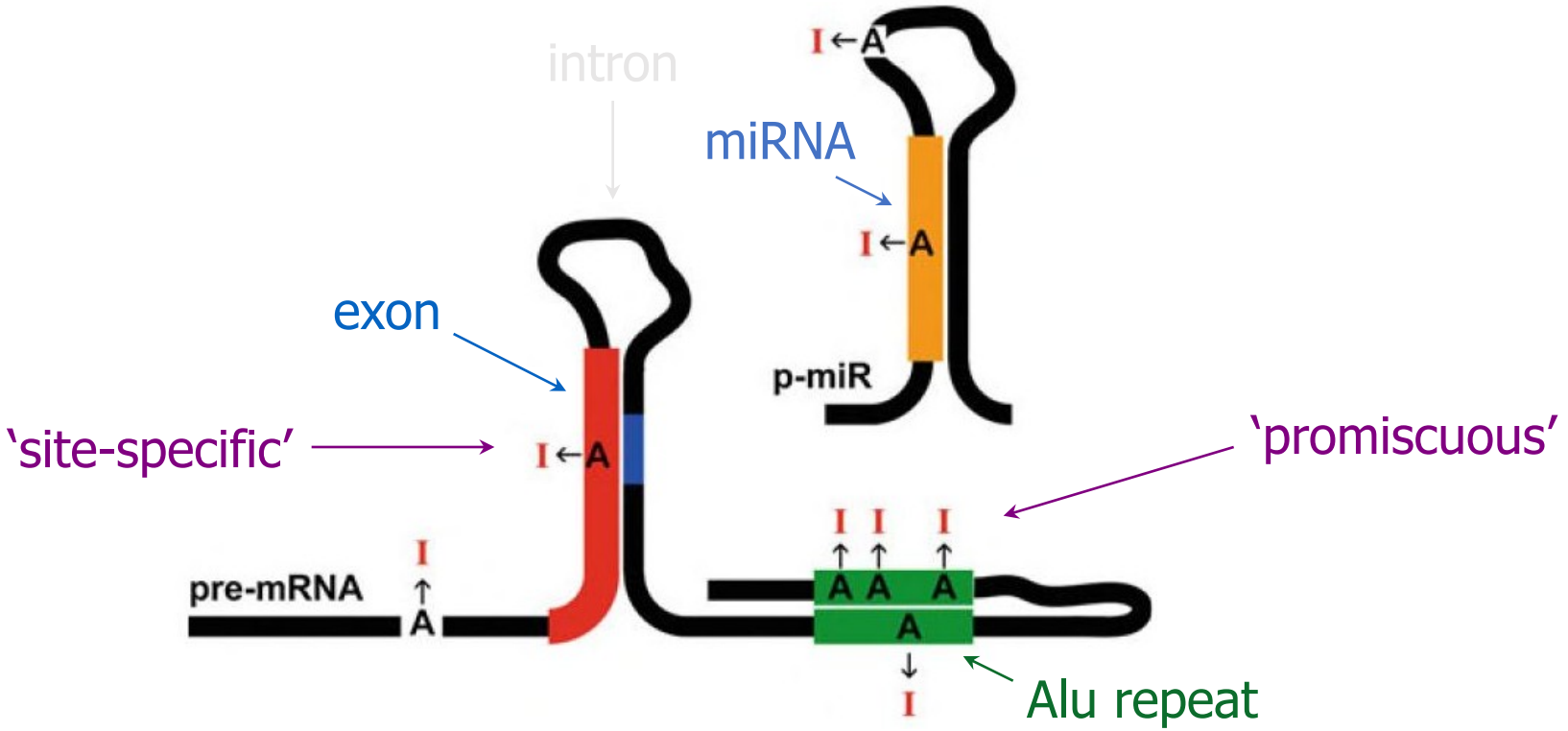
Liam Keegan

CEITEC

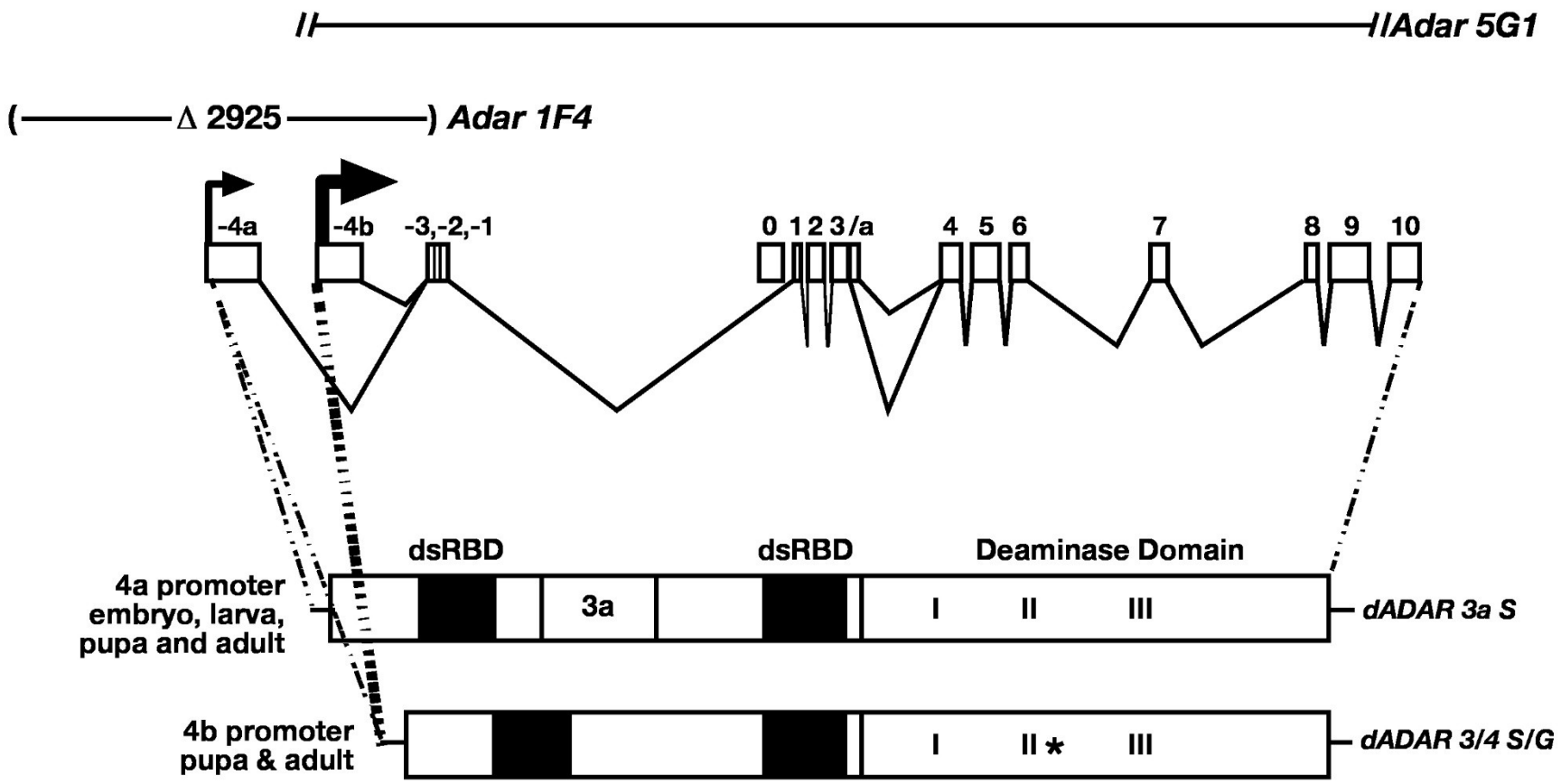
Conversion of adenosine to inosine



Adenosine Deaminases acting on RNA (ADARs) edit A → I in RNA



Drosophila Adar mutations on Chr X*:*
Adar ^{1F4} is a hypomorph and *Adar* ^{5G1} is a null.



Pallidino *et al.* Cell 2000

High RNA editing in transcripts with exon sequence conservation.

Name	Protein function	Number of sites	Functional consequence*	• % editing at each site
	Voltage-gated ion channels			
DSCl	Na^+ channel	1	+++	• 50%
Ca-alpha1T	Ca^{2+} channel	1	+	• 30%
DmCa1D	Ca^{2+} channel	5	++	• 30, 100, 95, 95, 100%
$\alpha_2\delta$	Ca^{2+} channel accessory subunit	3	+	• 80, 50, 50%
Shaker (*Sh*)	K^+ channel	6	+++	• 10, 10, 50, 50, 80, 50%
ether-a-go-go (*eag*)	K^+ channel	6	+++	• 50, 100, 20, 90, 10, 80%
slowpoke (*slo*)	K^+ channel	2	+	• 90, 90%
	Synaptic release machinery			
Synaptotagmin (*syt*)	Ca^{2+} sensor	4	+++	• 5, 50, 50, 100%
Dunc-13	SNARE binding	1	++	• 40%
Stoned B (*stnB*)	?	1	+	• 90%
complexin (*cpx*)	SNARE protein	3	?	• 50, 20, 30%
lap	Adaptor protein	1	?	• 10%
	Ligand-gated ion channels			
Dα5	nAChRα subunit	7	+++	• 80, 80, 100, 50, 30, 60%
ARD	nAChRβ subunit	4	?	• 50, 90, 50, 100%
SBD	nAChRβ subunit	2	+++	• 30, 20%
Resistance to dieldrin (*Rdl*)	GABA-receptor	6	+++	• 15, 80, 90, 90, 20, 10%

Gene class	Symbol	cDNA	Protein	Residue change[a]	3′-UTR[b]	Molecular function
Vesicular traffic	*Rab26*	GH21984	CG7605-PA	K365R		GTPase (Yoshie et al. 2000)
	Rlip	GH01995	CG11622-PA	I229V, E230G, K233E, E254G, K265R	2478, 2480, 2482, 2486, 2740	Ral GTPase activator (Jullien-Flores et al. 2000)
	rab3-GEF	HL01222	CG5627-PA	Q2022R, S2054G	1306	Rab guanyl-nucleotide exchange factor (Lloyd et al. 2000)
	endoA	GH12907	CG14296-PA	K129R, K137E		Promotes synaptic vesicle budding (Verstreken et al. 2002)
	α-Adaptin	RH30202	CG4260-PA	T207A		Component of endocytosis, subunit of AP-2 (Gonzalez-Gaitan and Jackle 1997)
	syd	GH19969	CG8110-PA	S983G		Kinesin-dependent axonal transport (Bowman et al. 2000)
Ion homeostasis	*Cpn*	GH08002	CG4795-PB	S402G		Ca^{+2} sequestration (Ballinger et al. 1993; Martin et al. 1993)
	Nckx30C	HL01989, GH04818	CG18860-PC	K365R		K^+-dependent Na^+, Ca^{2+} antiporter (Haug-Collet et al. 1999)
	CG1090	GH23040	CG1090-PA	L357L, S358G, L387L		K^+-dependent Na^+, Ca^{2+} antiporter (Webel et al. 2002)
	Atpα	GH23483	CG5670-PD	Y390C		Na^+, K^+ exchanging ATPase (Palladino et al.2003)
	CG32699	HL01250	CG32699-PA	I175M		Ca^{+2} binding, acyltransferase activity[c]
Signal transduction	*Mob1*	RH70633	CG11711-PD	N91D		Activator of Trc kinase (He et al. 2005)
	boss	GH10049	CG8285-PA	T529A, T533A		G-protein-coupled receptor (Reinke and Zipursky 1988; Kramer et al. 1991)
Ion channel	*SK*	GH16664	CG10706-PD	Y377C	2788	K^+ channel (Kohler et al. 1996)
	CG31116	GH23529	CG31116-PA	K232R, T259A, K268R, E269G		Cl^- channel (Jentsch et al. 2005a)
Cytoskeletal components	*spir*	GH13327	CG10076-PC	K339R		Actin nucleation factor (Quinlan et al. 2005)
	Atx2	GH01409	CG5166-PA	K320R, K337R		Regulator of actin filament formation (Satterfield et al. 2002)
	CG32245	GH04632, GH25458	CG32245-PB	R296R, N297D	2225, 2248, 2249, 2656	Structural constituent of cytoskeleton[c]
Other	*CG32809*	GH23439	CG32809-PB	K179R		ATP binding[c]
	retm	GH05975	CG9528-PA	Q245R		Phosphatidylinositol transporter[c]
Unknown	*CG1552*	GH14443	CG1552-PA	K121R		None
	CG31531	GH25780	CG31531-PB	K679E		None
	CG3556	GH17087	CG3556-PA	I572V		None
	CG9801	GH23026	CG9801-PA	S345G		None
	l(1)G0196	GH02989	CG14616-PC	Q1148R, S1172G, Q1176R		None[d]
	CG12001	HL01040	CG12001-PA	I325V		None
	CG30079	HL05615	CG30079-PA	I127M, T303A, Q343R, Q358R, S360G		None

Drosophila edited transcripts

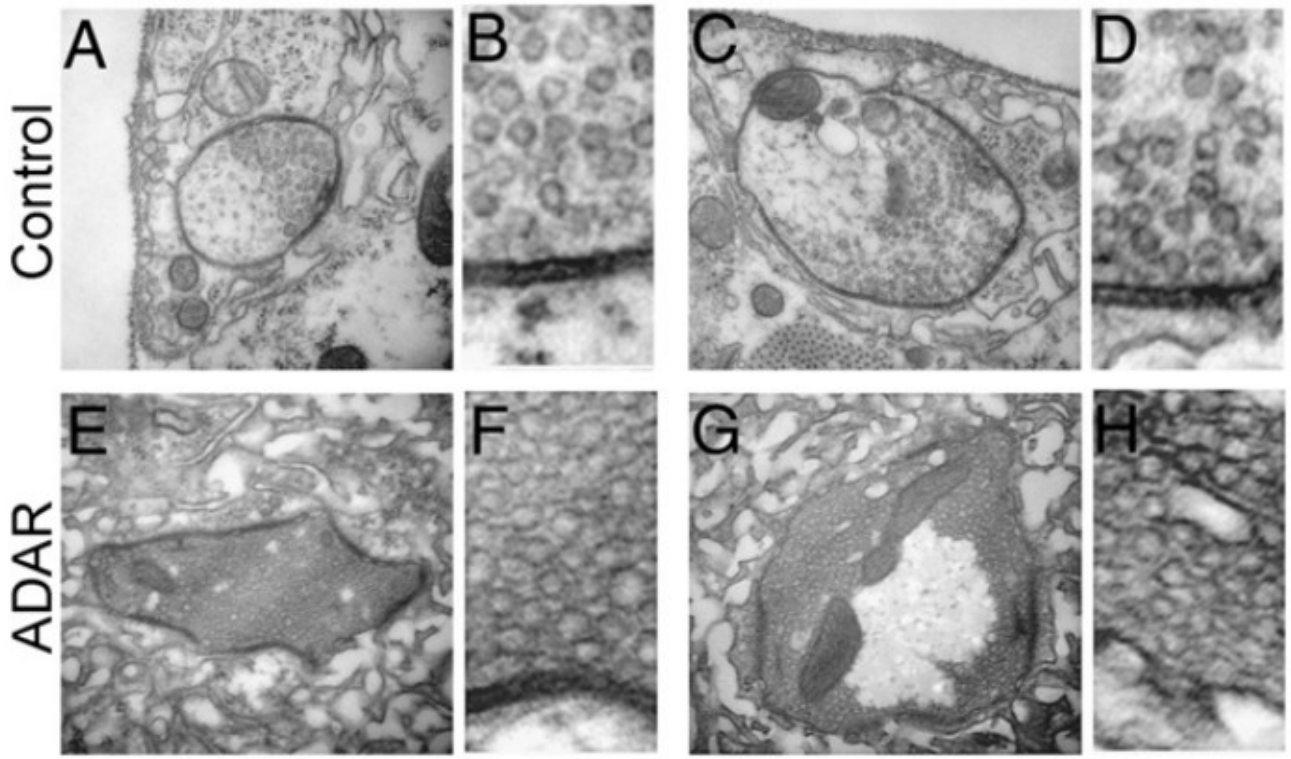


Fig. 5. Ultrastructural analysis shows that vesicle number, distribution and density are altered in *Adar* mutants. (A and C) Representative examples of control synaptic boutons. Note that parts of the bouton contain synaptic vesicles while some areas are devoid of vesicles. (B and D) show a higher magnification of a portion of the bouton ultrastructure presented in A and C respectively. (E and G) Representative examples of $Adar^{5G1}$ mutant synaptic boutons. Note that, in E, the synaptic bouton is entirely covered by vesicles. In G, while the central part of the bouton is devoid of vesicles, its entire circumference is packed with vesicles. Both E and G show a large number of vesicles compared to controls. The density of the vesicles present in the mutant is increased compared to controls. (F and H) show a higher magnification of a portion of the bouton ultrastructure presented in E and G respectively. The vesicles present in F and H are much more densely packed than vesicles observed in B and D.

ARTICLE

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ADAR-mediated RNA editing suppresses sleep by acting as a brake on glutamatergic synaptic plasticity

J.E. Robinson[1,2], J. Paluch[3], D.K. Dickman[3] & W.J. Joiner[1,2,4,5]

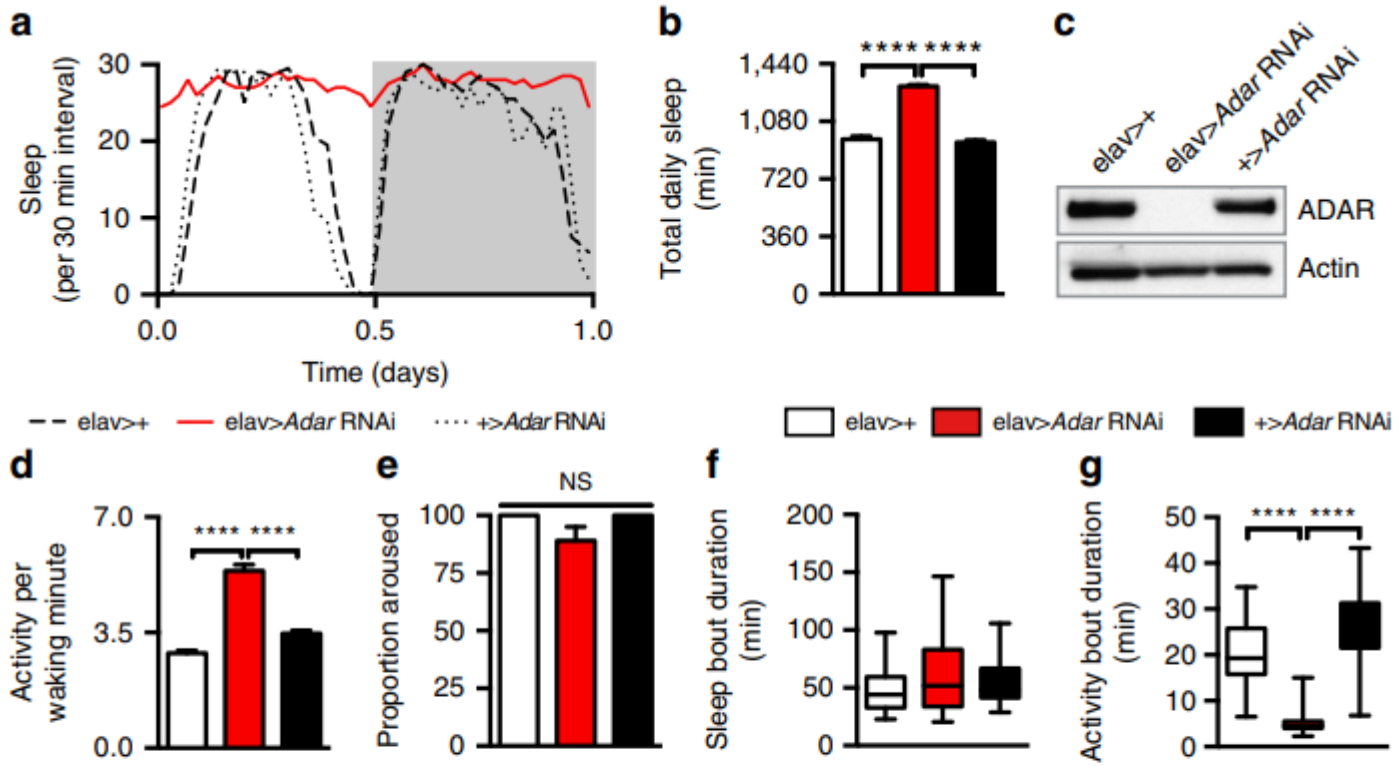

Figure 1 | *Adar* stabilizes the waking state to suppress sleep. (**a**) Representative sleep profiles of male elav>*Adar* RNAi and controls. (**b**) Quantification of sleep in **a**. Pan-neuronal knockdown of *Adar* increases sleep in elav>*RNAi* animals relative to controls. (**c**) Representative western blot of fly brains indicates efficient knockdown of ADAR expression in elav>*Adar* RNAi flies. (**d**) Waking activity is not reduced in elav>*Adar* RNAi animals. (**e**) Sleep in elav>*Adar* RNAi animals is acutely reversible by mechanical perturbation. (**f**) Sleep maintenance is unaffected in elav>*Adar* RNAi animals. (**g**) The wake state is destabilized in elav>*Adar* RNAi animals relative to controls. For each panel: *elav*>+ ($n=39$); elav>*Adar* RNAi ($n=54$); +>*Adar* RNAi ($n=39$). For all figures *, **, *** and **** indicate $P<0.05$, 0.01, 0.001 and 0.0001, respectively, and error bars represent s.e.m.

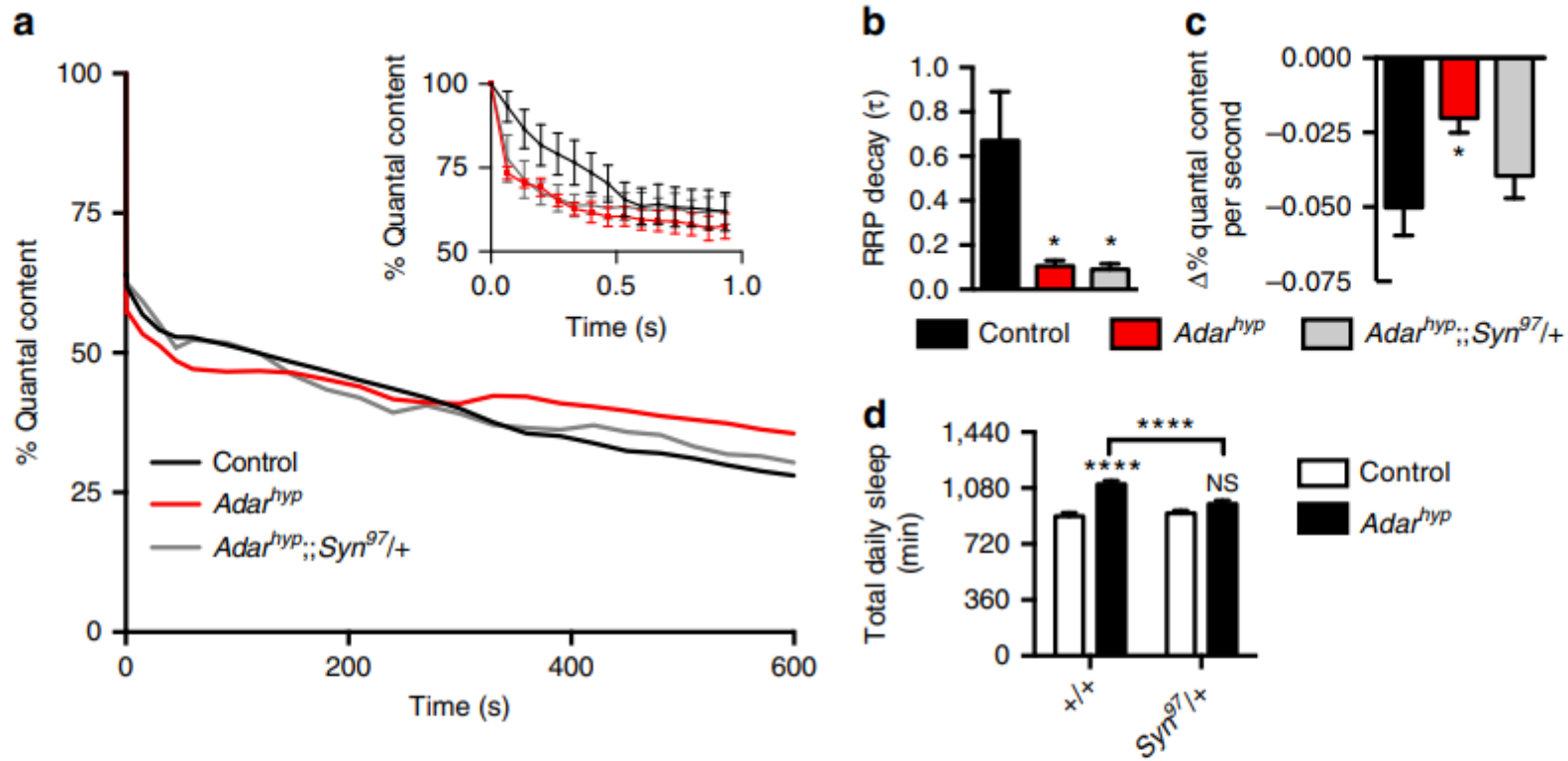

Figure 6 | Reversing the expansion of the reserve vesicle pool restores normal sleep to *Adar* mutants. (**a**) High frequency (15 Hz) stimulation at the NMJ causes a faster rate of depletion of the RRP (inset) and a slower rate of depletion of the RP (main figure) in $Adar^{hyp}$ mutants relative to controls. Heterozygous loss of *Synapsin* only rescues changes in the reserve pool (control ($n=8$); $Adar^{hyp}$ ($n=6$); $Adar^{hyp};;Syn^{97}/+$ ($n=6$)). (**b**) Quantification of the decay rate of the RRP in **a**. Tau values are based on an exponential fit to the change in quantal content over time, ending at 1.0 s (control ($n=8$); $Adar^{hyp}$ ($n=6$); $Adar^{hyp};;Syn^{97}/+$ ($n=6$)). (**c**) Quantification of the decay rate of the reserve pool. Values are based on linear regression fit of changes in quantal content over time, beginning at 60 s (control ($n=8$); $Adar^{hyp}$ ($n=6$); $Adar^{hyp};;Syn^{97}/+$ ($n=6$)). (**d**) Heterozygous loss of *Synapsin* restores normal sleep to *Adar* mutants (black bars) without altering sleep in controls (white bars; $n=45$–48 for each genotype). In **a**–**d**, control refers to animals harbouring wild-type *Adar* and wild-type *Syn* alleles in the same w^{1118} genetic background as both mutants.

Reading

- Mainly the Flybook review on sleep from Genetics.
- The mushroom body review is also good.

ANNUAL REVIEWS

Circadian Rhythms and Sleep in *Drosophila melanogaster*

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The *Drosophila* Mushroom Body: From Architecture to Algorithm in a Learning Circuit

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