Review

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.

[Supplemental material is available for this article.]

To survive and thrive in challenging environments, the brain is constantly comparing the past and the present trying to predict the future. Doing so efficiently requires the nervous system to filter and integrate important information about the environment and the animal's physiological state on a moment-to-moment scale to adapt instantaneous and future behavior.

What happens in our brains when we are hungry, thirsty, or tired? And where and how is information about the external world integrated to modulate behavior according to our needs? Research on state-dependent plasticity in the insect brain has provided important insights to these questions. In addition to its well-known role in associative learning and memory, the mushroom body (MB) acts as a dynamic integrator of past and current (multi)sensory experience with physiological and behavioral states, and thereby dynamically influences a wide range of essential behaviors. In the past decade, the MB has been anatomically and functionally dissected to a single-cell level and our understanding of the circuit mechanisms underpinning behavioral adaptation has advanced immensely (Aso et al. 2014; Owald et al. 2015; Li et al. 2020a).

The general logic of the MB is composed of three layers. The intrinsic layer comprises the cholinergic Kenyon cells (KCs), which form five different lobes with their axons (γ, α/β, and α′/β′) and can be further subdivided into nine subtypes according to more detailed morphological and molecular analysis (see Figs. 1 and 2). The output layer includes about 20 MB output neurons (MBONs), which are GABAergic, glutamatergic, or cholinergic. The input layer predominantly contains dopaminergic neurons (DANs), but also different aminergic and peptidergic circuitries (Riemensperger et al. 2005; Mao and Davis 2009; Pech et al. 2013; Perisse et al. 2013; Aso et al. 2014; Yang et al. 2016; Li et al. 2020a; Scaplen et al. 2021). Here there are, at least at first approximation, two parallel systems in place: one conveying negative value (via PPL1 DANs) and the other conveying positive value (via PAM DANs) (Schwaerzel et al. 2003; Aso et al. 2012; Burke et al. 2012; Liu et al. 2012; Huetteroth et al. 2015; Yamagata et al. 2015). Associative memories or concurrent experience induce short- or long-term changes in synaptic weight at the output level, shifting the system either toward approach or avoidance behavior (Séjourné et al. 2011; Bräcker et al. 2013; Plaçais et al. 2013; Bouzaiane et al. 2015; Hige et al. 2015; Ichinose et al. 2015; Lewis et al. 2015; Owald et al. 2015; Perisse et al. 2016; Felsenberg et al. 2017, 2018; Berry et al. 2018; Handler et al. 2019; Zhang et al. 2019; Bilz et al. 2020; Li et al. 2020a; Ichinose et al. 2021; McCurdy et al. 2021; Villar et al. 2022).

Sophisticated behavioral assays, as well as advanced in vivo preparations for detailed multiphoton or fast whole-brain imaging, now allow for further unprecedented insights into behavioral and physiological adaptations in the behaving fly. In this review, we discuss recent important findings of the circuit mechanisms enabling the MB to detect and integrate important needs of the body such as eating, sleeping, and mating, thereby modulating sensory-driven decisions depending on the internal state or goals

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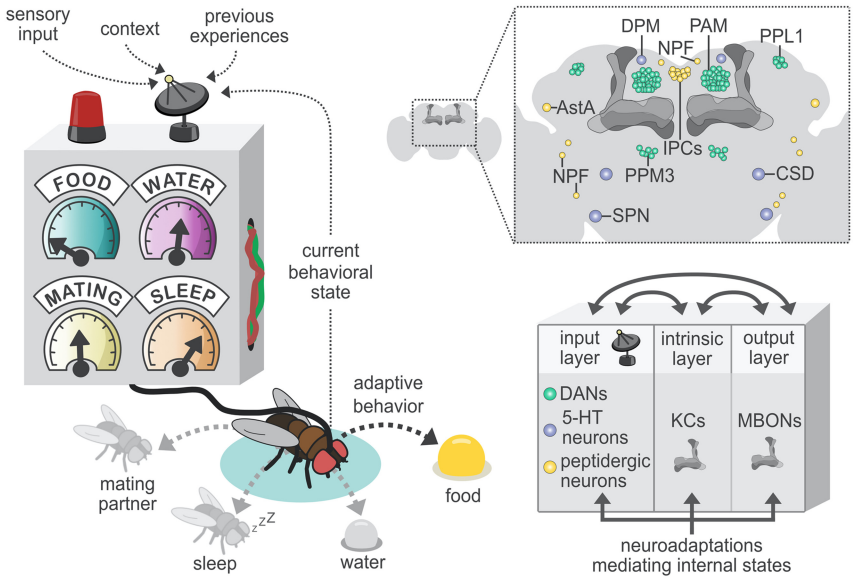

Figure 1. The MB as a panel control of body states. (*Left*) The MB integrates sensory information about the environment and the current context and behavioral state to regulate sensory valence according to the physiological needs of the fly in a moment-to-moment basis. As different internal states are 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.

of the animal. While such state-dependent signals can act at all three layers, most of the state signals appear to affect DANs activity and thereby, change how positive and negative signals are conveyed—or not—to the MB (Krashes et al. 2009; Bräcker et al. 2013; Lin et al. 2014; Cohn et al. 2015; Lewis et al. 2015; Tsao et al. 2018; Siju et al. 2020; Zolin et al. 2021). However, other inputs to the MB can also be targeted by state-dependent modulation, such as serotoninergic (Keene et al. 2006; Haynes et al. 2015; Ries et al. 2017; Turrel et al. 2018; Scheunemann et al. 2019) and octopaminergic signals (Sayin et al. 2019; Kobler et al. 2020; Hermanns et al. 2022), as well as plasticity parameters directly at the KC or MBON level (Plaçais et al. 2017; Handler et al. 2019; Lee et al. 2021). Here, we review these and other state-dependent changes and the potential mechanisms by which they may affect behavior. We further discuss how these adaptations correlate with the current behavioral state of the animal and with long-lasting maladaptive states such as addiction and depression. By doing so, we highlight the role of the MB and connected circuits as a tightly regulated control hub of internal states in the fly brain (Fig. 1).

The social brain

The mushroom body modulates reproductive state-dependent choices and memories

Reproduction is a potent drive causing fundamental adaptations in animal behavior for finding suitable mating partners, executing successful copulation, and ensuring the fitness of the offspring. Naturally, this involves distinct adaptations in males and females. While hormones and neuromodulators act at several levels (e.g., olfactory and auditory pathways) (Auer and Benton 2016; Sayin et al. 2018), the convergence of external and internal signals at the MB circuit might allow for the optimization of goal-directed behavior in different contexts. At the level of the MB, changes in the mating state are integrated mainly at the dopamine input layer (Keleman et al. 2012; Azanchi et al. 2013; Zhao et al. 2018; Siju et al. 2020, 2021; Boehm et al. 2022), subsequently routing behavioral output by transiently or constantly changing plasticity at KC to MBON synapses.

Mating experience changes how males and females perceive their environment and members of their own species. One evident example of how mating status affects fly behavior is the transition of previously receptive females into a long-lasting state of rejection of copulation after the first mating encounter (Connolly and Cook 1973; Dickson 2008; Rezával et al. 2012). This, in turn, affects male fly courtship behavior by reducing courtship attempts toward mated females, presumably to avoid futile attempts and waste of resources. A key neuromodulator underlying this behavioral change is the sex pheromone 11-*cis*-Vaccenyl acetate (cVA) produced by males and transferred to females during mating (Butterworth 1969; Ejima and Griffith 2007; Datta et al. 2008; Griffith and Ejima 2009; Everaerts et al. 2010; Keleman et al. 2012; Montague and Baker 2016; Lim et al. 2018). In males, specific DANs (aSP13 and PAM cluster) lastingly change their response magnitude to future cVA encounters, which is integrated via the dopamine receptor 1 (Dop1R1) in downstream KCs and aversion-mediating MBONs (Keleman et al. 2012; Montague and Baker 2016). Such an experience-dependent change in response to pheromones could, in part, also induce female rejection behavior toward males after the first mating encounter. In line with this idea, mated females show a reduced response to cVA at the level of the antennal lobe (AL; DA1 glomerulus) (Lebreton et al. 2014). Although it is not understood how this could translate into persistent changes in cVA responses across MB layers, mating-dependent alterations in cVA responses in aversion-mediating MBONs might be a common feature in adapting male and female behavior according to mating state (Lebreton et al. 2014; Boehm et al. 2022).

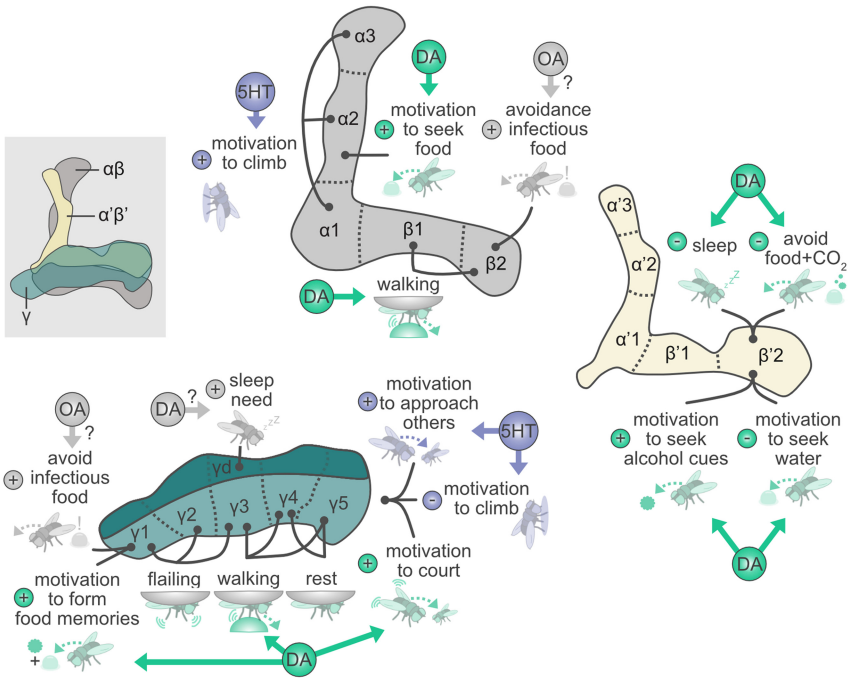

Figure 2. Complex integration of neuromodulatory signals by the MB mediates labile and enduring behavioral changes. Neuromodulators such as dopamine (DA), serotonin (5HT), and octopamine (OA) are signaled to specific MB lobes, allowing for representations of the behavioral state and behavioral adaptations. DAN and MBON innervations divide the MB lobes into 15 compartments and each type of DAN and MBON projecting to a specific compartment is named accordingly. The same compartment and circuits can integrate different signals to regulate distinct responses (e.g., γ5 mediating changes in climbing behavior and motivation to court in response to dopamine and serotonin, respectively). The modulation of different needs and behaviors by interconnected or common circuits might facilitate complex regulatory interactions (e.g., changes in activity in β′2 regulating arousal might affect the consolidation of ethanol memories). Arrows not linked to any compartment in the γ lobe denote not known compartment assignment. For simplicity, some KC subtypes are not shown. Based on data from Krashes et al. (2009), Keleman et al. (2012), Bräcker et al. (2013), Cohn et al. (2015), Musso et al. (2015), Sitaraman et al. (2015a,b), Perisse et al. (2016), Ries et al. (2017), Sayin et al. (2019), Senapati et al. (2019), Kobler et al. (2020), Scaplen et al. (2020), Sun et al. (2020), and Zolin et al. (2021). Please note that representative examples are shown due to space constraints. For a more detailed overview of behaviors, states, and circuits involved in different behavioral adaptations, see Supplemental Table S1.

Apart from inducing changes in mating behavior, cVA-dependent plasticity in DANs (β′1) might trigger an adaptation in odor-mediated food choices of females after mating. In virgins, avoidance-inducing PPL1 DANs (e.g., PPL1-α2α′2) reduce the olfactory preference for a highly nutritious food component, polyamine. Upon mating, females are attracted to polyamine odor through neuropeptidergic modulation of polyamine-sensitive olfactory sensory neurons and increased activity of PAM-β′1 DANs, which in turn reduces polyamine aversion through β′2 MBONs (Hussain et al. 2016a,b; Boehm et al. 2022). This behavioral adaptation also comprises the other higher-order olfactory center, the lateral horn (LH). In mated females, PPL1 DANs no longer counteract polyamine attraction, possibly through the modulation of MBON-α2sc and its direct connection to specific LH neurons.

In addition to dopamine, octopamine in males was shown to be important for female rejection-mediated courtship suppression (Zhou et al. 2012). Specific octopamine neurons (OANs) in the suboesophageal zone (SEZ, a region functionally similar to the mammalian brain stem) are both necessary and sufficient for cVA-dependent memory formation via octopamine receptors (OAMB) in KCs (Zhou et al. 2012). Interestingly, octopamine also mediates aggressive behavior toward potential male rivals (Zhou et al. 2008). Thus, the detection of cVA by OANs appears to suppress courtship in response to an encounter with a mated female eliciting aggressive behavior in the presence of another male (Wang and Anderson 2010). How these two behaviors are individually rooted and coordinated within the MB or other higher brain structures needs to be further investigated.

Ensuring the fitness and survival of the progeny also underlies highly adaptive processes. Interestingly, the MB seems to have a default inhibitory function on egg laying, since ablation of the MB increases egg laying even in virgin flies (Fleischmann et al. 2001). When a female *Drosophila* is in the course of egg laying, her sensory perception and decision-making undergo changes optimizing her reproductive success. Flies use fermenting fruit as a food source and as sites for oviposition, therefore, ethanol, acids, amines, and similar odors are predictors of high-quality egg laying grounds (Azanchi et al. 2013; Hussain et al. 2016a, b). On the other hand, geosmin (Stensmyr et al. 2012), an alarming smell indicating rotten food contaminated with certain bacteria, is highly aversive. Opposing DAN inputs to the MB (via PPL1/PAM and PPM3) reinforcing aversion and attraction behavior, respectively, are responsible for driving the behavioral choice of an optimal ethanol concentration (Azanchi et al. 2013). Such a mechanism could represent a universal design principle of the dopaminergic reinforcing system determining optimal conditions for reproductive behavior (e.g., for feeding, mating, and egg laying). Additionally, the LH may have a key role in selecting the optimal egg laying substrate, highlighting the multilayered integration of state-dependent behavioral adaptation (Chin et al. 2018).

Importantly, state-dependent plasticity in higher-order structures not only allows for adaptive behavioral choices, but also gates memory processes (Scheunemann et al. 2018). For example, virgin female *Drosophila* suppress consolidation of aversive olfactory long-term memory, via default inhibition of a pair of serotonergic neurons (SPNs) upstream of the avoidance-promoting DAN-MB

memory circuit. After mating, phosphodiesterase activity in SPNs is reduced, which disinhibits their activity via increased cAMP signaling and promotes the consolidation of aversive memories (Scheunemann et al. 2018). This may be critical for fitness, as blocking long-term storage of aversive associations might engage flies in higher risk-taking behavior and promote foraging to find mating partners, while developing risk-averse behavior after mating could ensure the survival and safety of the progeny. Such a behavioral shift in response to reproductive state is commonly observed in mammals including humans (Pattwell et al. 2013, 2016); the neurobiological underpinning, however, is not well understood.

An intriguing aspect of MB modulation of behavior is its ability to integrate multiple sensory modalities; the detailed mechanisms are the subject of active investigation (Thiagarajan et al. 2022; Okray et al. 2023). For instance, the sensory stimuli driving DAN responses during egg laying decisions are multisensory, coming mainly from olfactory and gustatory but also undetermined sensory input (Chin et al. 2018). This cross-modal integration allows the MB to combine olfactory cues with other sensory inputs, such as gustatory and visual information. This integrated sensory processing could be crucial for making comprehensive reproductive decisions, as it enables the female to assess not only the suitability of potential mates but also the quality of egg laying sites and to form context-dependent memories for future behavior. Moreover, it will be interesting to decipher how multisensory input to important courtship-controlling neurons (e.g., P1/pC1) is integrated with MB processing (Clowney et al. 2015; Deutsch et al. 2020; Jung et al. 2020; Cheriyamkunnel et al. 2021; Shen et al. 2023).

The mushroom body brings flies together

Although flies mostly interact with each other to mate and compete for mating partners, recent evidence indicates that they are capable of simple types of sociability beyond these purposes. Indeed, *Drosophila* shows approach behavior to other individuals and a natural tendency to aggregate (Jiang et al. 2020; Sun et al. 2020). This moderate degree of sociability seems to be beneficial for flies, as it facilitates the spread of relevant information about their surroundings, learning, and avoidance of dangers within a group (Kacsoh et al. 2015; Ramdya et al. 2015; Muria et al. 2021; Wu et al. 2023).

Since group behavior and social interactions beyond mating could be critical for increasing the likelihood of survival and fitness, it is conceivable that the *Drosophila* brain contains defined circuits that regulate the drive to approach conspecifics according to previous social experience. This notion of a social drive is strengthened by studies showing the impact of lack of social experience on following social interactions and on the regulation of other internal drives. For instance, after being in isolation for 3–6 days, flies typically increase their activity levels, the frequency of social encounters, and the number of aggressive behaviors (Liu et al. 2018; Bentzur et al. 2021). This isolation-induced brain state appears to be long-lasting and characterized by a decreased need to sleep and overconsumption of food. These effects involve changes in NPF (homolog of NPY) signaling, a known input to MB-projecting DANs (PPL1-γ1-pedc) (Ganguly-Fitzgerald et al. 2006; Li et al. 2021).

Apart from regulating the need to mate, the MB has been shown to be required for social learning such as copying other flies' mating preferences and avoidance of laying eggs in dangerous environments (Kacsoh et al. 2015; Nöbel et al. 2023). Recent evidence further indicates that this neuropil integrates visual and olfactory cues associated with social experience for generating the motivation to interact. Specifically, the activity of the γ lobe has been shown to be sensitive to such cues promoting social motivation and approach behavior (Fig. 2; Sun et al. 2020). Here, serotonin signaling from dorsal paired medial (DPM) neurons, which are involved in MB-dependent memory (Keene et al. 2006), is required for the expression of social drive (Sun et al. 2020). Interestingly, this serotonergic cluster also responds to putative social cues such as CO_2 released by other individuals, thereby modulating the activity of α/β KCs to boost memory expression in a social context (Muria et al. 2021). These results stress the role of the MB and associated neurons as integration centers for social cues that regulate the drive of flies to be with others and other key functions of the brain according to the social environment.

Interestingly, previous research with flies indicates that a lack of social cues affecting future encounters and other internal drives can alter the sensitivity to pheromones and dopamine (Fernandez et al. 2017; Agrawal et al. 2019; Sethi et al. 2019). Bearing this in mind, it is conceivable that deprivation of social experience may also have long-lasting effects on the transcriptional landscape and activity levels of socially relevant subsets of MB and associated neurons. The extent to which such changes in neuron activity could impact information processing within the MB and its contribution in influencing or controlling relevant brain states more globally remain to be seen.

The maladaptive brain

Depression- and addiction-like states are influenced by the mushroom body

Brains contain a complex regulatory machinery that, when chronically dysregulated, may lead to maladaptive behavioral states that are typical of addiction and depression. Given the important role of the MB in balancing avoidance and approach based on state and experience, what is its role in the induction and maintenance of states of unhealthy motivation?

Similar to mammals, flies exposed to lasting and uncontrollable stress such as repeated vibrations, social defeats and deprivation (e.g., from food or sleep) fall into a persistent depression-like state characterized by a low motivation to seek natural rewards and avoid punishments (Yang et al. 2013; Ries et al. 2017; Araujo et al. 2018; Kim et al. 2018). This brain state also leads to cognitive deficits, pessimistic biases, increased aggression, and reduced drive to perform innate behaviors such as climbing and mating (Araujo et al. 2018; Deakin et al. 2018; Jia et al. 2021).

Not surprisingly, transitions in and out of this depression-like state are associated with different levels of activity within the MB and the fly's reward system. Supporting the notion that aminergic systems are key mediators of motivational states, enduring stress has been shown to reduce brain levels of dopamine and serotonin (Araujo et al. 2018). For example, chronic exposure to vibrations reduces serotonergic input from DPMs to KCs in the α lobe, which dampens the activity of these neurons and the motivation to climb (Ries et al. 2017). The depressive behavior can be alleviated by enhancing serotonergic signaling artificially or by exposing flies to natural rewards such as sweet sensation (Ries et al. 2017; Hu et al. 2020). Interestingly, this relief from sugar experience is mediated by rewarding PAM DANs, which respond to octopamine-mediated sweet signals and activate DPMs via α/β KCs, setting serotonin release to the α-lobes back to the levels that are required to promote climbing (Fig. 2; Hermanns et al. 2022). Although the activity of DANs such as PPL1-γ1-pedc gates the formation of aversive stressor associations (Kim et al. 2018), their output has also been shown to underlie the development of learning deficits after chronic stress, likely by leading to abnormal spontaneous activity in downstream MB neurons (Jia et al. 2021).

Contrary to depression, addiction leads to a long-lasting internal state characterized by a high motivation to seek the reward triggered by drugs and their cues even in the face of aversive consequences (Vanderschuren and Everitt 2004). The susceptibility to this state seems to be conserved from humans to flies, as

Drosophila not only finds drugs of abuse and its cues rewarding, but also develops features of addiction such as voluntary self-administration, craving, withdrawal, and relapse (Devineni and Heberlein 2009; Kaun et al. 2011; Robinson et al. 2012; Ghezzi et al. 2014; Highfill et al. 2019; Kanno et al. 2021).

Critical for the maintenance of this state is the formation of appetitive memories of cues that are associated with drugs, as it facilitates seeking behavior and repeated exposure. Moreover, although still largely ununderstood, recent data suggest that the formation of associations related to opioids also influences the effects of the drug itself, leading to functional tolerance only when they are consumed in the context previously linked with drug intake (Hou et al. 2023). Just as preference to natural rewards such as sugar and water, the consolidation and expression of conditioned preference to drugs like alcohol requires the MB and dopaminergic input (Kaun et al. 2011; Scaplen et al. 2020; Kanno et al. 2021). A key difference is that alcohol experience does not rely on a specific subset of DANs but engages a broader population of PAM DANs, which could underlie the high motivational value of this substance. For instance, the encoding of positive alcohol associations requires the activity at the population level of PAM DANs, which increases with prolonged exposure (Scaplen et al. 2020). It has been proposed that this broad activation could lead to a highly stable memory and promote compulsive cue-seeking despite punishment by antagonizing the function of aversive DANs (Scaplen et al. 2020; Jovanoski et al. 2023). Once consolidated, the expression of positive alcohol memories is mediated by the activity of PPL1-α′2α2 and PAM-β′2a DANs projecting to the vertical and horizontal MB lobes, respectively (Scaplen et al. 2020).

Interestingly, the susceptibility of flies to display addiction-like behaviors seems to be influenced by past experiences or other motivational drives. For example, an increase of NPF levels that signal reward after mating interferes with the ability of flies to form rewarding alcohol associations, while NPF decreases after social rejection and promotes consumption (Shohat-Ophir et al. 2012). Importantly, the formation of positive alcohol associations and its progressive ingestion coincides with long-lasting alterations in gene expression and splicing in MB neurons. For example, the levels of Dop1R1 in β, β′, and γ lobes increase over days of self-administration, correlating with an escalation of alcohol intake and preference. Alcohol experience is also thought to induce functional changes in Dop1R2 in the MB via alterations in isoform expression (Petruccelli et al. 2018, 2020; Kanno et al. 2021). Suggesting common long-lasting effects of drug abuse, cocaine ingestion also alters the expression of genes associated with signal transduction at the KC level (Baker et al. 2021). Other neuroadaptations reported in mice after drug exposure, such as increases in mitochondrial trafficking in DANs, appear to be relevant in the dopaminergic system of *Drosophila* for forming positive alcohol associations (Knabbe et al. 2022). Although not yet investigated, similar long-lasting plastic changes across synapses between the MB and reward circuitry could be triggered by exposure to stressors and contribute to the development and maintenance of depressive-like states.

Future work with *Drosophila* will shed light on how MB plasticity, internal state, and context might facilitate entries and exits from these states of unbalanced motivation.

The hungry brain

Homeostasis and how it changes valence coding in the mushroom body

The processing of sensory information varies widely across behavioral states. Although olfaction is the most studied sensory modality for the MB circuit, also gustatory, temperature, mechanosensory (including proprioceptive), auditory, and visual inputs are integrated and may underlie state-dependent modulation (Hallem et al. 2006; Hong et al. 2008; Benton 2009; Gerber et al. 2009; Bang et al. 2011; Vogt et al. 2014, 2016; Kirkhart and Scott 2015; Sachse and Beshel 2016; Li et al. 2020b; Siju et al. 2020; Devineni et al. 2021). Metabolic states have been shown to strongly influence plasticity in the MB circuit mainly at the dopaminergic input level affecting both innate and learned behavior (Krashes et al. 2009; Bräcker et al. 2013; Lin et al. 2014; Tsao et al. 2018).

When flies interact with their environment and encounter specific sensory stimuli, the MB allows for the association between these stimuli and various outcomes or experiences. This is, for instance, crucial for recognizing food sources or avoiding dangers (Heisenberg 2003; Akalal et al. 2006; Owald and Waddell 2015; Cognigni et al. 2018; Mariano et al. 2020; Felsenberg 2021). Arguing against a strict functional division of memory processes and instinctive responses, work in the last decade demonstrated that the MB also regulates innate behaviors such as avoiding odors or cold temperatures according to the internal homeostatic needs (Hong et al. 2008; Bräcker et al. 2013; Siju et al. 2014; Lewis et al. 2015; Owald et al. 2015; Shih et al. 2015; Perisse et al. 2016; Sayin et al. 2019; Lin 2023). For example, hunger motivates flies to approach food odors, with DANs driving innate food odor-seeking through multiple MBON types (Tsao et al. 2018; Sayin et al. 2019). The MB also helps hungry flies modulate innate aversion, possibly as a gate for risk-taking behavior (Bräcker et al. 2013). For instance, while fed flies are strongly repelled by a mixture of the highly aversive odor CO_2 and food odor vinegar, hungry flies show reduced aversion to it (Bräcker et al. 2013). This behavioral change is achieved by a vinegar-mediated activation of specific hunger-modulated PAM DANs (β2 and β′2), which suppress the output of avoidance-triggering MBONs (Bräcker et al. 2013; Lewis et al. 2015). Starvation also modulates odor-evoked responses by disinhibition of other MBONs, for instance at the MB heel (y1) (Fig. 2; Perisse et al. 2016). The metabolic state-dependent modulation of odor responses of MB-innervating DANs is mediated by several neuromodulators in response to satiety and hunger signals. To date, identified hunger signals include (short) neuropeptide F (NPF and sNPF) and serotonin while satiety signals are mainly conveyed by insulin and AstA (Krashes et al. 2009; Tsao et al. 2018). These studies and additional work suggest that DANs can redirect behavioral choices in food-deprived flies by modulating the synapse between KCs and MBONs (Bräcker et al. 2013; Plaçais and Preat 2013; Musso et al. 2015; Plaçais et al. 2017). Such a parallel mechanism, together with the LH, would ensure both hardwired and adaptive responses as needed, for instance, in hunger states.

In general, such state-dependent modulations can cause a change in the strength of the phasic DAN response to sensory input like odors (Lewis et al. 2015; Perisse et al. 2016; Jovanoski et al. 2023), but also changes tonic and spontaneous DAN activity (Ichinose et al. 2017). This is most visible in two MB-projecting DANs (PPL1-γ2′α1 and PPL1-γ1-pedc), which show spontaneous activity like slow oscillations in fed and water-sated flies. Their spontaneous activity is blocked by hunger (PPL1-γ1-pedc) and thirst (PPL1-γ2α′1) (Krashes et al. 2009; Plaçais et al. 2012; Plaçais and Preat 2013; Senapati et al. 2019), which is thought to increase motivation to seek food and water and to change the MB circuit to allow flies to be attracted to odors associated with these rewards during memory retrieval (Fig. 2; Krashes et al. 2009; Lin et al. 2014). Interestingly, studies training flies by pairing an odor with the optogenetic activation of subsets of DANs indicate that flies can form short-term sugar memories independent of the metabolic state with the input of specific PAM DANs (β′2 and γ4) to the MB (Huetteroth et al. 2015). Other PAM DANs (γ5) are sensitive to nutrient signals and form long-lasting food-seeking memories. Importantly, consolidation of such a sugar-rewarded memory trace is gated by increased ongoing activity of PPL1-γ1-pedc (Musso et al.

2015; Pavlowsky et al. 2018), the same DAN whose activity is blocked by nutrient deficiency. Thus, PPL1-γ1-pedc behaves like a state-dependent gatekeeper for inducing long-term changes to odor valence. In line with the idea of reward and punishment neurons, it seems that signals from food intake are conveyed by specific "rewarding" DANs (PAM DANs) while deficiency states for metabolites are integrated via changing spontaneous activity patterns in "punishment" DANs (PPL1 DANs). This could imply that the degree of inhibition from PPL1 gates a persistent behavioral change dependent on the perceived value of sugar-rewarded odors.

Likewise, water deprivation states are integrated by inhibition of specific "punishment" DANs (PPL1-γ2α′1), but also by inhibition of a subset of "rewarding" DANs (PAM-β′2a) via lateral horn leucokinin (LHLK) neurons and leucokinin signals (Senapati et al. 2019). The first is likely serving a deficiency detection mechanism similar as observed for food deprivation, while inhibition of PAM-β′2a might promote specificity for thirst rewards by blocking sugar reward processing in these neurons. Interestingly, leucokinin is a shared signal for both hunger and thirst and drives severely starved flies to approach food-associated odors through the activation of approach-promoting DANs. Activation of PAM-β′2p and PAM-β′2m and disinhibition of PPL1-γ2α′1 and PAM-β′2a DANs via additional neuromodulators override thirst-induced inhibition. Thus, fine-tuned modulation of the dopaminergic system allows flies to adapt behavior to the most relevant cues, while its malfunction can lead to compulsive behavior as described in the section above (Jovanoski et al. 2023).

Taken together, the DAN layer can bidirectionally modify behavior according to homeostatic needs through modulation of the synaptic weight of KC to MBON connections or directly by instructing the output layer. This function of DANs is mediated primarily by two dopamine receptors, inhibitory D2R and Dop1R2 (Sayin et al. 2018, 2019). Interestingly, the latter is linked to a second messenger pathway (PKCδ) that controls mitochondrial function and thereby energy fluxes that fuel memory processes (e.g., trigger protein synthesis–dependent long-term memory) (Comyn et al. 2023).

State-dependent plasticity occurs also directly at the level of MBONs (e.g., via direct input from octopamine and serotonin). Octopamine was shown to signal a successful food encounter directly at the MB output level to override and break starvation-dependent food-seeking behavior (Sayin et al. 2019). Serotonin was found to modulate specific output neurons in response to water deprivation, which supports the consolidation of water-reward learning (Lee et al. 2021). Interestingly, serotonin also modulates hunger state-dependent attraction and aversion toward odors at the first olfactory center, the AL (Vogt et al. 2021). A recent study showed that thirst-dependent modulation of gene expression is pronounced in glia. Interestingly, these cells modulate odor responses in a thirst-dependent fashion via tripartite synaptic connections with glutamatergic MBONs (γ5) and downstream neurons (Park et al. 2022). Together, while DAN activity appears to be the most prominent side of state-dependent modulation, KCs and MBONs are also direct targets of adaptive mechanisms, mediated fundamentally by glia-dependent signals.

Interestingly, protein synthesis–dependent aversive LTM is also sensitive to metabolic states and an increased frequency in the Ca^{2+} signals of "energy-signaling" PPL1-γ1-pedc DAN is indispensable for memory consolidation (Plaçais et al. 2012, 2017; Musso et al. 2015). Recent advances in the understanding of metabolic pathways in MB neurons demonstrated that the energy demands of neurons involved in memory formation are fueled by metabolites from glia cells surrounding the MB. In detail, it was shown that in the fed state, the formation of protein synthesis–dependent long-term memory relies on the regulation of both pyruvate (a glucose derivative) metabolism in MB neurons (Plaçais et al. 2017) and glucose metabolism in glial cells (de Tredern et al. 2021). When flies are starved, LTM formation is blocked, which is beneficial for surviving food restriction (Mery and Kawecki 2005; Plaçais and Preat 2013). This adaptive plasticity is specific to LTM, as starved flies maintain their ability to form consolidated—but protein synthesis–independent—memory fueled by ketone bodies from glia (Silva et al. 2022). Together, this demonstrates that (1) memory formation, in general, is sensitive to energy restriction, (2) energy transfer from the glia to the MB is regulated by metabolic state, and strikingly (3) increased energy flux in MB mitochondria can trigger the formation of stable protein synthesis–dependent LTM instead of a labile fast-decaying memory.

Finally, dietary adaptations like an increase in food consumption or preference for specific nutrients have been well described in female *Drosophila* in response to mating (Ribeiro and Dickson 2010; Vargas et al. 2010; Laturney et al. 2023). The MB might contribute significantly to such a crossover integration of different states (e.g., mating and nutrient homeostasis) (Hussain et al. 2016a; Boehm et al. 2022).

In summary, the MB is highly sensitive to metabolic deprivation states, ensuring the increased value of food-related sensory input is signaled only in contexts when physiological need is high and behavior has to be adapted to counteract the imbalance of metabolic homeostasis.

Movement and foraging: the mushroom body responds to behavioral states

Work over the past decades revealed that, apart from integrating external and homeostatic signals, the MB also responds to behavioral states of the body such as walking, resting, or flailing. Such moment-to-moment representation of locomotion and movement in DANs and MBONs might allow the brain to integrate the animal's ongoing behavior into decision-making, goal-directed behavior, and learning (Martin et al. 1998; Brembs 2009; Cohn et al. 2015; Aimon et al. 2019, 2023; Siju et al. 2020; Zolin et al. 2021).

Increased locomotion can be a consequence of state-dependent adaptations (e.g., in response to starvation) mediated by various neuromodulators (prominently dopamine) that, among other brain regions, are known to modulate MB plasticity (Lee et al. 2004; Eriksson et al. 2017; Lin et al. 2019). Although locomotion is vital for hungry animals to find food sources, it can also be a waste of already low-energy stores. Thus, the effort an animal invests into reaching a goal such as food must be tightly regulated. A deeper analysis of individual foraging sequences (like initiation and active search or engagement and disengagement of food sources) is providing a more detailed view of how DAN-driven motivational states control behavioral initiation and persistence and mediate the transition between tasks (Sayin et al. 2019; Zolin et al. 2021).

Ongoing dopamine release during locomotion might engage the same synaptic plasticity mechanisms as those driving memory formation, allowing both rewards and self-generated actions to modulate and reinforce behavior. For instance, dopamine versus octopamine feedback at the level of KCs and the MBON-γ1-pedc>αβ regulates persisting versus stopping food odor tracking, suggesting a tight regulation of the length and intensity of goal-directed behavior (Sayin et al. 2019). On its way to a goal, a fly might form new associations, potentially engaging similar DAN-dependent plasticity mechanisms to tune current as well as future behavior (Fisher et al. 2022). Interestingly, a similar, compartmentalized activity of DANs in the MB γ-lobe is triggered by electric shock or sugar ingestion and flailing or rest, respectively (see Fig. 2), suggesting that the animal's behavioral state could act as an additional reinforcement stimulus (Cohn et al. 2015). The combination of ongoing DAN activity during odor pursuit and rapid dopamine-dependent plasticity in the MB γ-lobe provides a potential mechanism for storing a permissive

trace of the fly's recent actions. This mechanism may present evidence of history-dependent odor-tracking behavior or facilitate the pursuit of an odor plume (Sayin et al. 2019; Zolin et al. 2021; Fisher et al. 2022; Aso et al. 2023). Notably, other DANs innervating the other MB lobes also show walk-induced activity to different degrees (Siju et al. 2020; Aimon et al. 2023). Interestingly, DANs innervating certain compartments (e.g., γ3) increase activity before the animal starts to walk, consistent with a putative instructive role in behavior initiation (Aimon et al. 2023).

Work over recent years has revealed that locomotion and movement influence neural activity in many brain areas across many organisms (Busse et al. 2017; Kaplan and Zimmer 2020). Using whole-brain imaging with high temporal resolution, recent data describe global brain activity during free and forced walk in *Drosophila* (Aimon et al. 2019, 2023). As for other animals, *Drosophila* brain activity is widely correlated with locomotion representing a global change in brain state. These results challenge the assumption that most of the DAN activity is related to decision-making, top-down motor control, or prediction error detection from sensory feedback and instead provide evidence that walk itself and somatosensory bottom-up stimuli are largely responsible for the observed activity patterns (Aimon et al. 2023). Interestingly, while DAN activity increases with locomotion, specific serotonin signals are strongly inhibited when the fly engages in walking (Aimon et al. 2023), reminiscent of a functional discrimination between go and no-go signals by dopamine and serotonin in mammals (Desrochers et al. 2022).

Regarding the role of the MB in the brain-behavior loop, population and whole-brain imaging data in behaving animals, together with the whole-brain electron microscope (EM) connectomes for *Drosophila*, provide novel, highly complementary, resources for generating new hypotheses. This will help addressing the question of how goal-oriented, seemingly purposive, and spontaneous movements are governed by explicit and implicit reinforcing signals from the DAN system and account for statistical learning in a natural environment.

The brain under alert

Innate immune signaling triggers mushroom body–dependent behavioral adaptation

An animal's survival hinges not only on its ability to find sufficient food but to avoid harmful sources and remember that they caused sickness. Pathogens introduced through food navigate through the digestive system, inciting an immune response. Besides instigating direct antimicrobial measures like the production of antimicrobial peptides (AMPs), harmful microbes also prompt behavioral adjustments and memory formation. This is crucial for minimizing the impact of infection and preventing recurrent exposure to the same or similar contaminated food sources. These adaptive behaviors, which include conditioned taste aversion (CTA) of tastes associated with malaise, are widespread across the animal kingdom. Nevertheless, the mechanisms governing CTA or pathogen-modulated feeding behaviors remain uncertain, particularly concerning the necessary communication between the body and the brain.

Important work in honeybees and *Caenorhabditis elegans* suggests a role for serotonin in integrating negative postingestive signals during the formation of CTA (Zhang et al. 2005; Wright et al. 2010), while evidence from *Drosophila* points to the involvement of octopamine and immune signaling in the brain in the context of pathogen-modulated behavior (Babin et al. 2014; Kurz et al. 2017; Schretter et al. 2018; Masuzzo et al. 2019; Kobler et al. 2020). Importantly, Kobler et al. (2020) implicated the fly MB and proposed a model in which the intake of ecologically relevant pathogenic bacteria in flies induces CTA through innate immune signaling in OANs, KCs, and specific MBONs, directly linking pathogen ingestion, the immune system, and the behavioral adaptations regulated by the brain. Flies lacking the innate immune receptor PGRP-LC in the nervous system, specifically in OANs, lose their ability to differentiate between harmless and pathogenic bacteria postingestion while naive flies are strongly attracted to the odors of these pathogens. The additional requirement for the "synaptic plasticity" gene encoding the calcium-sensitive dependent adenylate cyclase rutabaga and synaptic output from β′2 and γ5 MBONs, indicates that the aversion to feeding on pathogenic bacteria involves an MB-dependent associative learning mechanism (Fig. 2). Moreover, flies lacking the important olfactory receptor Orco do not adapt their behavior and continue to consume pathogenic bacteria, suggesting an association between the bacterial odor and a negative postingestive experience.

PGRP-LC-mediated signaling in OANs, and possibly an involvement of other organs such as the fat body in conveying negative postingestion signals (Y Wang and IC Grunwald Kadow, unpubl.), indicate how complex neuromodulatory input from the body to the MB guides behavioral adaptations upon pathogen ingestion. Narrowing down the specific scenario that leads to the activation of PGRP-LC signaling in OANs is important. One hypothesis is that bacterial components or metabolites might traverse the gastrointestinal tract epithelium, activating PGRP-LC directly in the nervous system. Alternatively, bacterial fragments (e.g., peptidoglycans, PGN) could cross the epithelial barrier, reach the hemolymph, and activate immune responses in other organs that are detected by the brain through secondary signals.

A major output of PGRP signaling is the production of AMPs. Intriguingly, some AMPs may also play roles as modulators in the central nervous system (Barajas-Azpeleta et al. 2018; Kobler et al. 2020). Two specific AMPs were shown to be essential for the formation of two types of memories, appetitive associative olfactory memory and memories of an unsuccessful mating experience (Barajas-Azpeleta et al. 2018). AMPs, such as Diptericin B (DptB), were induced in the heads of adult flies following behavioral conditioning, and the activity of DptB and another AMP, Gram-negative bacteria binding protein like 3 (GNBP-like3), proved essential in modulating these forms of memory. These findings suggest that certain AMPs might have been repurposed in the nervous system, functioning as neuromodulators during evolution. However, the precise mechanism through which these immune peptides influence long-term memory remains unclear at this stage.

PGRP-LC signaling, and therefore possibly AMPs, could be directly involved in synaptic plasticity in the MB. A comprehensive forward genetic screen aimed at identifying genes involved in homeostatic plasticity, primarily using synaptic electrophysiology at the neuromuscular junction (NMJ) as the main assessment method, revealed mutations within the PGRP-LC locus that impede presynaptic homeostasis (Harris et al. 2015, 2018). Based on their findings, the authors hypothesized that PGRP-LC might serve as a receptor for a retrograde signal responsible for mediating homeostatic signaling from muscle to nerve. Whether it plays a similar role in the MB remains to be seen but is an exciting possibility.

Taken together, the MB's role in integrating sensory information with delayed postingestion signals from organs such as the gut or the fat body, forming memories of food quality, presents an intriguing avenue for future research.

The sleepy brain

The mushroom body regulates sleep and arousal

As other animals, insects transition between a wake and a sleep state according to the time of the day, level of tiredness, and the

influence of other internal needs and environmental factors (Huber et al. 2004; Ganguly-Fitzgerald et al. 2006; Beckwith and French 2019; Shafer and Keene 2021). Sleep in flies can be defined as a period of quiescence marked by decreased responsiveness to the surroundings and characterized by changes in brain activity levels and electrical signatures relative to wakefulness (Hendricks et al. 2000; Shaw et al. 2000; van Alphen et al. 2013, 2021; Yap et al. 2017; Tainton-Heap et al. 2021). Although a unified theory of the function of sleep remains elusive, the last two decades of sleep research in *Drosophila* have provided further evidence of its key role in memory consolidation (Donlea et al. 2011; Dag et al. 2019; Lei et al. 2022; Marquand et al. 2023) and in recuperative processes of the brain such as synaptic homeostasis (Donlea et al. 2009; Gilestro et al. 2009; Bushey et al. 2011; Huang et al. 2020; Weiss and Donlea 2021). Since sleep and essential active behaviors such as foraging or mating are mutually exclusive, keeping the right balance between sleep and activity is critical.

To contribute to such balance, the MB comprises sleep- and wake-promoting KCs and MBONs and integrates inputs from different sleep-regulating circuits. These include signals from DANs activating glutamatergic wake-promoting MBONs (such as those innervating the γ, β, and β′ lobes) and GABAergic input from DPMs that promote sleep by inhibiting wake-promoting α′/β′-KCs (Fig. 2; Joiner et al. 2006; Pitman et al. 2006; Haynes et al. 2015; Sitaraman et al. 2015a,b; Driscoll et al. 2021; French et al. 2021). Once the fly has fallen asleep, the MB contributes to the state-dependent processing of salient olfactory information by promoting awakening in the presence of relevant stimuli (e.g., to food odors when hungry). This state-dependent sensory gate during sleep partly relies on an MBON in the β′2 and γ5 area that receives input from PAM DANs and signals to the sleep-regulatory fan-shaped body of the central complex (CC) (French et al. 2021).

An important hallmark of sleep that is conserved from flies to humans is its tight regulation by homeostatic drive that generates need to sleep according to the time spent awake and the complexity of visual inputs processed throughout the day (Borbely 1982; Ganguly-Fitzgerald et al. 2006; Donlea et al. 2009; Borbély et al. 2016; Donlea et al. 2017; Kirszenblat et al. 2019). When the need to sleep is high, awake flies are in a tired state in which important functions of the brain like attention and MB-dependent memory consolidation are compromised (Ganguly-Fitzgerald et al. 2006; Seugnet et al. 2008; Li et al. 2009). During this state, specific networks in the CC start displaying sleep-like state properties like slow wave activity, which promotes sensory decoupling and sleep need signaling (Raccuglia et al. 2019, 2022; Hasenhuetl et al. 2024). This finding indicates that tiredness is experienced at a network level, a notion supported by local sleep events during the wake in mammals that affect circuit function and lead to task-specific impairments (Vyazovskiy et al. 2011; Nir et al. 2017; Quercia et al. 2018; Suárez-Grimalt and Raccuglia 2021). Reminiscent of these local sleep events, KCs seem to go "offline" after a prolonged time awake, showing reduced spontaneous and evoked activity and being less likely to respond consistently to stimuli (Bushey et al. 2015). Although the signaling of the need to sleep has been mostly attributed to circuitries associated with the CC and to neurons in the ventral nerve cord (Donlea et al. 2014; Seidner et al. 2015; Liu et al. 2016; Blum et al. 2021; Ho et al. 2022; Jones et al. 2023), defined compartments of the MB are known to participate in the propagation of homeostatic signals. Specifically, sleep loss induces the activity of homeostatic recovery or rebound sleep by triggering the activity of a sleep-promoting KC–MBON circuit comprised of γd KCs and corresponding MBONs and reducing the activity of a wake-promoting one (Fig. 2; Sitaraman et al. 2015a).

Given the overlap between sleep-regulating and associative learning MB circuits (Keene et al. 2006; Claridge-Chang et al. 2009; Liu et al. 2012), learning-induced changes in these pathways might contribute to the build-up of tiredness or facilitate transitions to sleep. This might promote the regulation of sleep based on learning experience and the stabilization of specific memories. For example, the activity of the excitatory MBON-γ2α′1 has been recently shown to be sensitive to the duration of courtship learning and to trigger postlearning sleep via a CC circuit after an intense learning experience (Lei et al. 2022). This could not only be relevant for supporting the consolidation of these associations but also favor the downscaling of the synapses that were highly engaged in the task (Bushey et al. 2011; Diering 2023). Apart from facilitating memory storage by promoting sleep, the MB must also allow for the consolidation of memories during internal states in which falling asleep is not the best option (e.g., when starving). To support this, hunger signals after training mediated by NPF neurotransmission trigger the activity of a sleep-independent memory circuit composed of γ1 DANs, MBONs, and α′/β′m KCs. This pathway allows for memory consolidation with little postlearning sleep, unlike a different MB microcircuit comprised of γ2/α′1 DANs, MBON-γ2α′1, and α′/β′ap KCs that is activated when the fly is fed and that promotes sleep (Chouhan et al. 2021).

Despite the progress in the identification of the pathways transmitting homeostatic information, it is still not understood how all these signals are integrated to regulate sleep and how many circuits undergoing plastic changes upon sleep loss participate in signaling tiredness. Indeed, the increasing need to sleep as time awake passes is well reflected by brain-wide increases in the potentiation of synapses, which is evidenced by rising levels of the presynaptic protein Bruchpilot. Changing levels of this synaptic correlate of tiredness are required in a sleep need-encoding circuitry in the CC to regulate sleep according to time spent awake (Gilestro et al. 2009; Liu et al. 2016; Huang et al. 2020; Ho et al. 2022). At the level of the MB, KCs strongly contribute to the increase of Bruchpilot across different lobes, while presynaptic neurons such as DANs appear to be less sensitive to this form of presynaptic plasticity upon sleep loss (Weiss and Donlea 2021). Interestingly, KC to MBON synapses that are known to encode sleep-independent memories appear to display less changes in the number of synaptic connections as a result of sleep loss than those encoding sleep-dependent memories (Weiss and Donlea 2021). This opens the striking possibility that tiredness may not equally affect the function of all circuits and could perhaps spare the encoding function of some of them. Interestingly, recent evidence indicates that MBONs and DANs of distinct compartments involved in aversive and appetitive learning support memory at different times of the day (Dissel et al. 2024). This could potentially represent an additional strategy for preventing circuit damage, ensuring optimal circuit function and learning.

Future research on the mechanisms of sleep regulation in the MB will not only shed light on the underpinnings of tiredness signaling but also contribute to our understanding of the MB's role on memory formation during sleep–wake transitions.

Conclusion and outlook

In this review, we have featured the MB as a sophisticated control unit orchestrating the influence of a panel of diverse internal and behavioral states on goal-directed behavior, decision-making, and learning and memory by changing the valence of incoming sensory cues (for a list of selected publications covering each section, see Fig. 3; a brief summary of their main findings is in Supplemental Table S1). The functional parallels observed between the MB and mammalian brain regions such as the cerebellum and striatum underscore its intricate role in modulating instantaneous and future actions through the interactions of body needs and

	Publication	Title	MB circuitry
The social brain	Keleman et al. (2012)	Dopamine neurons modulate pheromone responses in *Drosophila* courtship learning	aSP13 DAN
	Azanchi et al. (2013)	Competing dopamine neurons drive oviposition choice for ethanol in *Drosophila*	α′β′ KCs PAM, PPM3 PPL1-γ1-pedc DANs
	Zhao et al. (2018)	Persistent activity in a recurrent circuit underlies courtship memory in *Drosophila*	γ KCs aSP13 DAN MBON-γ5β′2a
	Siju et al. (2020)	Valence and state-dependent population coding in dopaminergic neurons in the fly mushoom body	α3,β′1 DANs
	Boehm et al. (2022)	Dopamine-gated learning circuit underpins reproductive state-dependent odor preference in *Drosophila* females	β′1 DANs β′2 MBONs PPL1 DANs (e.g., α2α′2) LH neurons
	Zhou et al. (2012)	Molecular genetic analysis of sexual rejection: roles of octopamine and its receptor OAMB in *Drosophila* courtship conditioning	αβ KCs OANs
	Sun et al. (2020)	Social attraction in *Drosophila* is regulated by the mushroom body and serotonergic system	DPMs γ KCs
	Muria et al. (2021)	Social facilitation of long-lasting memory is mediated by CO_2 in *Drosophila*	DPMs α′β′, αβ KCs
	Wu et al. (2023)	Aversive conditioning information transmission in *Drosophila*	α′β′, γ KCs MBON-α′1 MBON-α′3 MBON-γ1γ2 MBON-γ4γ5
The maladapted brain	Kaun et al. (2011)	A *Drosophila* model for alcohol reward	γ, α′β′,αβ KCs
	Ries et al. (2017)	Serotonin modulates a depression-like state in *Drosophila* responsive to lithium treatment	α KCs (+ climbing) β KCs (- climbing)
	Petruccelli et al. (2018)	Alcohol activates Scabrous-Notch to influence associated memories	KCs, MB-wide changes
	Scaplen et al. (2020)	Circuits that encode and guide alcohol-associated preference	PAM-β′2a, PPL1-α2α′2 DANs MBON-α′2 MBON-β′2mp MBON-β2β′2a
	Hermanns et al. (2022)	Octopamine mediates sugar relief from a chronic-stress-induced depression-like state in *Drosophila*	OANs β,β′ γ PAM DANs
Movement / foraging	Cohn et al. (2015)	Compartmentalized neuromodulation shapes sensory processing in *Drosophila*	γ KCs γ2-5 PAM DANs γ2-5 MBONs
	Siju et al. (2020)	Dopamine modulation of sensory processing and adaptive behavior in flies in a mushroom body-compartment-specific manner	DANs innervating all compartments
	Zolin et al. (2021)	Context-dependent representations of movement in *Drosophila* dopaminergic reinforcement pathways	γ KCs γ2-5 PAM DANs
	Aimon et al. (2023)	Fast near-whole-brain imaging in adult *Drosophila* during responses to stimuli and behavior	brain-wide chages, pronounced in γ3 DANs
	Eriksson et al. (2017)	Neuromodulatory circuit effects on *Drosophila* feeding behaviour and metabolism	broad effects, including in PPL1 DANs

	Publication	Title	MB circuitry
The hungry brain	Krashes et al. (2009)	A neural circuit mechanism integrating motivational state with memory expression in *Drosophila*	PPL1-γ1-pedc DAN
	Bracker et al. (2013)	Essential role of the mushroom body in context-dependent CO_2 avoidance in *Drosophila*	all KCs (especially α′β′) β′2 DANs biVPN
	Lewis et al. (2015)	A higher brain circuit for immediate integration of conflicting sensory information in *Drosophila*	
	Tsao et al. (2018)	*Drosophila* mushroom bodies integrate hunger and satiety signals to control innate food-seeking behavior	PPL1-α3 DAN>MBON- α3 β2β′2α′β′2 -DAN>MBON-β2 β′2α PPL1-α′2α 2>MBONα′2 α2 PPL1-γ2 α′1>MBON-γ2 α′1 PPL1-γ 1-pedc>MBON- γ1-αb>MBON-α1β′
	Sayin et al. (2019)	A neural circuit arbitrates between persistence and withdrawal in hungry *Drosophila*	αβ KCs PPL1-γ1-pedc MBON-γ1-αβ MBON-α2sc
	Perisse et al. (2016)	Aversive learning and appetitive motivation toggle feed-forward inhibition in the *Drosophila* mushroom body	PPL1-γ1-pedc MBON-γ1-αβ
	Placais et al. (2013)	To favor survival under food shortage, the brain disables costly memory	PPL1-γ1-pedc
The brain under alert	Barajas-Azpeleta et al. (2018)*	Antimicrobial peptides modulate long-term memory	Head fat body potential MB involvement
	Babin et al. (2014)*	Fruit flies learn to avoid odours associated with virulent infection	Potential MB involvement
	Kobler et al. (2020)	Immune receptor signaling and the mushroom body mediate post-ingestion pathogen avoidance	γβ KCs MBON-β′2mp_ bilateral, MBON-γ5β′2a OANs
	Copf et al. (2011)	Cytokine signaling through the JAK/STAT pathway is required for long-term memory in *Drosophila*	all KCs
The sleepy brain	Sitaraman, Aso, Rubin et al. (2015)	Control of sleep by dopaminergic inputs to the *Drosophila* mushroom body	PAM, PPL1 DANs MBON-γ5β′2a/ β′2mp_bilat. MBON-γ2α′1
	Sitaraman, Aso, Jin et al. (2015)	Propagation of homeostatic sleep signals by segregated synaptic microcircuits of the *Drosophila* mushroom body	γm, α′β′KCs MBON-γ5β′2a/ β′2mp /β′2mp_bilat. (+ wake) γd KCs MBON-γ2α′1 (+ sleep, sleep need signaling
	Weiss and Donlea (2021)	Sleep deprivation results in diverse patterns of synaptic scaling across the *Drosophila* mushroom bodies	KCs > MBONs, DANs, APL and DPMs
	Chouchan et al. (2021)	Availability of food determines the need for sleep in memory consolidation	α′β′m KCs, PPL1-γ1-pedc MBON-γ1-pedc (hunger) α′β′ap KCs, PPL1-γ2-α′1 DAN and MBON-γ2-α′1 (fed)
	Lei et al. (2022)	A neural circuit linking learning and sleep in *Drosophila* long-term memory	MBON-γ2α′1 MBON-β′2mp

Figure 3. Selected publications for state-dependent modulations in the MB. Each physiological or behavioral state reviewed in this article for its role in modulating plasticity in the MB and changing behavioral output is represented by four to nine publications. Please note that these publications are selected to give a broad overview covering most of the diverse lines of research. The studies marked with an asterisk indicate that the involvement of the MB was not tested and remains to assessed. For a brief summary of the key findings of these studies, see Supplemental Table S1. (MB) Mushroom body, (KCs) Kenyon cells, (MBONs) mushroom body output neurons, (DANs) dopaminergic neurons, (PAM DANs) protocerebral anterior medial dopaminergic neurons, (PPL1 DANs) protocerebral posterior lateral dopaminergic neurons, (OANs) octopaminergic neurons, (APL) anterior paired lateral neurons (GABAergic), (DPM) dorsal paired medial neurons (GABAergic and serotonergic), (NPF) neuropeptide-F neurons, (AL) antennal lobe, (LH) lateral horn.

states and dopaminergic signals (Strausfeld et al. 1998; Farris 2011; Valjent and Gangarossa 2021; Perisse et al. 2023).

The common integration of different body needs within MB circuits may be key for facilitating complex regulatory interactions and the expression of the most adaptive behavioral output at every given moment (Lebreton et al. 2017; Beckwith and French 2019). This output is constantly updated by past and present contextual cues that leave state-dependent plasticity traces within sensing circuitries and MB neurons (Fig. 1). While substantial progress has been made in understanding state-dependent changes in activity

within the MB network, the underlying mechanisms (e.g., varying input, pre- or postsynaptic adaptations) remain largely elusive. While we explored some adaptations such as alterations in presynaptic protein levels and cAMP pathway regulation (Tomchik and Davis 2009; Gervasi et al. 2010; Lee 2015; Scheunemann et al. 2018, 2019), other pre- and postsynaptic adaptations may contribute to state-dependent changes in activity (Zhang et al. 2019; Pribbenow et al. 2022; Davidson et al. 2023; Yamada et al. 2023). Additional fascinating avenues for future investigation include potential adaptations in the firing mode of neurons of the input layer in response to external or physiological signals (similarly to neurons encoding tiredness in the CC; Liu et al. 2016), and differential responses of downstream layers due to state-dependent changes in receptor expression and trafficking (Yamamoto and Seto 2014; Pribbenow et al. 2022). Bearing in mind that dopaminergic and other neuron types can release multiple neurotransmitters and are involved in promoting several behaviors (e.g., serotonergic and GABAergic DPMs promoting sleep and social approach) (Haynes et al. 2015; Aso et al. 2019; Sun et al. 2020; Yamazaki et al. 2023), it would be also interesting to explore potential context- and state-dependent alterations in cotransmission and their implications on behavior (Chen et al. 2023).

Despite tremendous progress in the understanding of MB function, it is still striking how MB neurons integrate such a complex and diverse array of information from changes in multisensory incoming signals. It is possible that different sensory inputs trigger plastic alterations in neurons of the input layer of distinct persistence and nature (e.g., changes in levels of activity, firing rates, cotransmitter release, or temporal coherence between dopaminergic, serotonergic, and other inputs), leading to specific plastic changes in downstream MB neurons. The coincidence of several signals that form a specific context boundary could further modify these factors, providing additional complexity to the array of the generated plastic changes and to the integration of inputs within the MB (Rodgers et al. 2011; Zeng et al. 2023).

Importantly, other brain areas receiving multisensory information described here in less detail, such as CC, AL, and LH, are known to contribute to the integration of and adaptation to body states and to behavioral organization (Pfeiffer and Homberg 2014; Sachse and Beshel 2016; Suárez-Grimalt and Raccuglia 2021; Vogt et al. 2021; Boehm et al. 2022; Sizemore et al. 2023). Considering the importance of generating robust responses to strong and salient stimuli, it is not surprising that parallel and partially redundant circuits may have evolved in the brain for the regulation of these outputs according to internal needs. In such a scenario, a potential key role of the MB might be to allow enduring state-dependent behavioral changes by undergoing long-lasting plastic synaptic reorganizations and supporting memory consolidation. Similar to the important role of synchronous activity between circuits and brain areas in memory consolidation (Plaçais et al. 2012; Miyamoto et al. 2016; Geva-Sagiv et al. 2023), the timing between the activity of MB neurons and that of other brain regions could also be a relevant factor for maintaining a unified representation of the fly's current state and induce a short- or long-term change in behavior. Future work will shed light on the intricate interplay of different brain circuits in contributing to the detection and generation of whole-brain states and their impact on the MB.

In conclusion, the *Drosophila* MB emerges as a multifaceted neuronal hub capable of decoding and integrating complex signals from internal and external environments. Unraveling the nuances of state-dependent plasticity at both the circuit and biochemical levels promises to deepen our understanding of adaptive behavior and cognition in this model organism and beyond. Future research should delve into additional plastic mechanisms and communication between MB and the body that allow efficient orchestration of behavioral responses by the MB.

Competing interest statement

The authors declare no competing interests.

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