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Review Article

Shared neurocircuitry underlying feeding and drugs of abuse in *Drosophila*

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ABSTRACT

The neural circuitry and molecules that control the rewarding properties of food and drugs of abuse appear to partially overlap in the mammalian brain. This has raised questions about the extent of the overlap and the precise role of specific circuit elements in reward and in other behaviors associated with feeding regulation and drug responses. The much simpler brain of invertebrates including the fruit fly *Drosophila*, offers an opportunity to make high-resolution maps of the circuits and molecules that govern behavior. Recent progress in *Drosophila* has revealed not only some common substrates for the actions of drugs of abuse and for the regulation of feeding, but also a remarkable level of conservation with vertebrates for key neuromodulatory transmitters. We speculate that *Drosophila* may serve as a model for distinguishing the neural mechanisms underlying normal and pathological motivational states that will be applicable to mammals.

In all animals, hunger drives the motivation to seek out food. Peripheral hormones directly regulate food seeking, and the targets of these peripheral hunger and satiety signals have been mapped to distinct hypothalamic and hindbrain nuclei in mammals [1]. Satiety signals and homeostatic brain circuits that limit feeding can be overridden by highly palatable food irrespective of the animal's nutritional state [2]. For example, remote manipulation of feeding circuits in mice and the fruit fly *Drosophila* promotes voracious eating in lieu of satiety signals [3,4]. In other words, organisms as distinct as mammals and invertebrates may have evolved common and hard-wired central feeding circuits in the brain.

Drugs of abuse have the capacity to evoke highly motivated and goal-directed behavior with an intensity that can eclipse

even that of a very hungry animal [5]. Addictive drugs such as cocaine and alcohol have reinforcing properties similar to food, and their pleiotropic actions are mediated in part by highly complex reward circuitry, such as the drug and feeding-engaged mesolimbic dopaminergic pathways [6]. Despite some commonalities in behavioral states and implicated brain circuitry, direct functional overlap of specific circuit elements has been difficult to prove, partly because of the ever-more appreciated complexity of the brain, but also because the quality and interpretation of behavioral measurements are rapidly improving [7].

Drosophila is an attractive model organism for conjoining behavioral, neuroanatomical, and genetic studies, because of its genetic tractability, the development of precise and high

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throughput assays, and the availability of tools to manipulate neuronal properties in a spatiotemporally accurate manner [8]. Remarkably, homeostatic metabolic systems and neurochemical circuit motifs in mammals and *Drosophila* appear to be largely conserved [9,10]. Circuit and neuron-specific manipulation in fruit flies has permitted the investigation of genetic and molecular targets that underlie the complex actions of addictive drugs as well as the homeostatic signals that regulate feeding [9,10].

Here, we review recent findings indicating that the regulation of feeding and the neural mechanisms of drugs of abuse in fruit flies may have significant overlap. We limit our scope to common neuromodulators and circuitry including dopamine, the amines tyramine and octopamine, the neuropeptide Y (NPY)-like neuropeptide F (NPF), the eight *Drosophila* insulin-like peptides (DILPs), and the neuropeptide corazonin. We include molecular and circuit-level descriptions for some drug-related behaviors that may be distinct from reward and motivation, but that appear to share some common elements with feeding. More comprehensive reviews on the regulation of feeding and on the molecular and behavioral actions of drugs of abuse in *Drosophila* were published recently [9,11,12].

Dopamine

Dopamine is a pleiotropic modulator of behavior in mammals and in fruit flies: depending on the behavioral context, dopamine in *Drosophila* affects sleep, mating, learning and memory, locomotion, feeding, and the effects of drugs of abuse [13–17]. There are approximately 280 dopaminergic neurons in the adult fly brain that are subdivided into eight major clusters based on their cell body location, and each cluster sends projections to distinct brain regions [Fig. 1] [18,19]. Dopamine signaling is detected by four receptors that are distributed broadly in the brain: the D1-like receptors DopR1 (DA1, DopR) and DopR2 (DAMB), the multiply spliced D2-like receptor D2R, and the DopEcR receptor that is also gated by the insect hormone ecdysone [20]. Emerging evidence indicates that particular dopamine clusters and even individual neurons likely form valence-specific circuit motifs that are engaged by conditioned or innate values of a stimulus, and whose function can be modified by the internal state [16,21–24].

Dopamine in feeding behaviors

Feeding behaviors are subdivided into six distinct phases: foraging/seeking, cessation of locomotion, meal initiation, consumption, meal termination, then finally food disengagement [11]. The feeding behaviors we discuss are complex and can overlap between two or more of the respective phases of feeding. A portion of our focus will encompass behavioral assays that assess goal-directed approach or avoidance behavior in the context of both unconditioned and conditioned food-related stimuli. The study of goal-directed approach or avoidance is a method to evaluate the relationship between valence-specific circuit motifs and innate/learned feeding motivation [16].

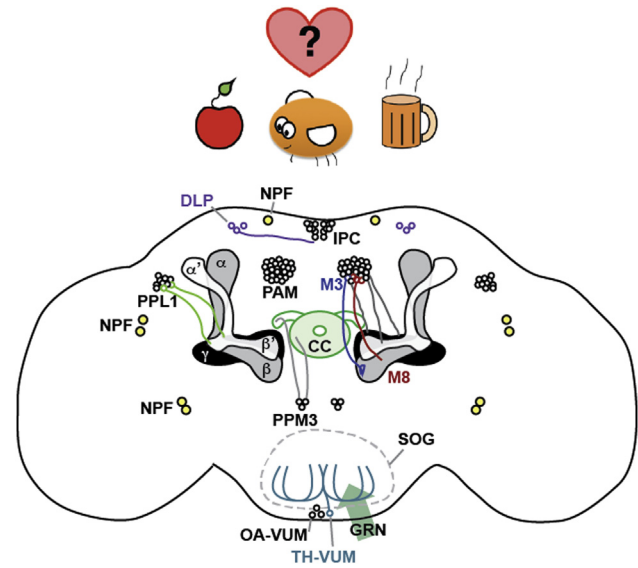


Fig. 1 – Schematic of the *Drosophila* adult brain. The diagram depicts the major neuropils and cell types discussed in this review, except for the mushroom body output neurons (MBON) that are excluded for purposes of clarity. All structures are bilaterally symmetric except for the ventral unpaired medial cells that are octopaminergic (OA-VUM) or dopaminergic (TH-VUM). Gustatory information is carried into the brain by gustatory receptor neurons (GRN) that terminate in the SOG. The TH-VUM makes an elaborate tree-like arborization in the SOG. The mushroom bodies are comprised of α/α' , β/β' , and γ lobes. The protocerebral anterior medial (PAM), protocerebral posterior lateral 1 (PPL1), and protocerebral posterior medial 3 (PPM3) clusters are all dopaminergic. The PAM and PPL1 neurons innervate distinct regions of the mushroom bodies and make both ipsilateral and contralateral (not shown) connections. The MBONs send dopamine/mushroom body information to protocerebral integration centers near the mushroom bodies. Individual PPM3 neurons innervate the ellipsoid body (doughnut) and fan-shaped body of the central complex (CC). The insulin-producing cells (IPC) of the pars intercerebralis neuroendocrine gland extend processes (not shown) medially to regions of the brain above the SOG and out of the brain to endocrine organs and other targets. The dorsal lateral protocerebral (DLP) cells express corazonin and extend processes to the IPC.

Protocerebral anterior medial neurons

Most fruit fly dopamine neurons, about 130 per hemisphere, are located in the protocerebral anterior medial (PAM) cluster. The PAM neurons densely innervate the mushroom bodies, prominent brain structures implicated in associative learning and memory and other behaviors. The mushroom bodies are composed of approximately 2500 Kenyon cells per hemisphere that are named α/α' , β/β' , and γ based on anatomical division [Fig. 1]. The PAM presynaptic terminals contact discrete regions in the β , β' , and γ lobes that comprise the

Table 1 – Tools used to manipulate specific neurons.

Type	Neuromodulator	Gal4 driver	Cells	Functions	Reference		
Biogenic amines	Dopamine	Ddc	All DA	Appetitive reinforcement Promotes ethanol preference Promotes ethanol reinforcement	[21,26,27]		
			TH	All DA and 12 PAM (MB-M3)	Aversive reinforcement Inhibits food intake Promotes ethanol-induced locomotor activity Promotes ethanol reinforcement Promotes odorant-induced appetitive behavior Promotes sucrose sensitivity	[14,17,21,22,26–34]	
				0273	130 PAMs	Appetitive reinforcement	[22,25]
				R58E02	90 PAMs	Appetitive reinforcement	[22,26]
				R48B04	55 PAMs	Appetitive reinforcement Promotes innate water seeking	[22]
				0104	40 PAMs	Appetitive reinforcement Promotes innate water seeking	[22,25,35]
				0279	M8 PAMs	Appetitive reinforcement	[35]
				NP5272	M3 PAMs	Aversive reinforcement	[28,33]
				NP1528	M3 PAMs	Aversive reinforcement	[33]
				NP0047	MB-MP1 MB-MV1	Aversive reinforcement	[28,36]
				NP2758	MB-MP1	Aversive reinforcement	[21,30,33]
				c061		Aversive reinforcement	[25,28,30,37]
				c259		Aversive reinforcement	[28]
				kra		Aversive reinforcement	[21,30]
				5htr1b	MB-MV1	Aversive reinforcement	[28]
				c346	PPM3	Promotes ethanol-induced locomotor activity Promotes ethanol preference	[14,21]
			Octopamine	NP7088	VUMs, AL	Sucrose sensitivity	[38]
				Tdc2	All	Appetitive reinforcement Promotes ethanol attraction Promotes odorant-induced appetitive behavior Promotes sucrose sensitivity	[22,25,34,39,40]
		Peptides	NPF	NPF		Appetitive reinforcement Inhibits alcohol preference Promotes food intake Promotes odorant approach Promotes odorant attraction Promotes odorant-induced appetitive behavior Promotes sucrose sensitivity Promotes willingness to overcome adversity	[4,17,30,41–45]
					Insulin-like peptide	DILP2	Food preference Inhibits innate appetitive behavior
DILP3	Food preference					[46]	
DILP4	Inhibits innate appetitive behavior					[44]	
Corazonin	Crz					Promotes food intake	[45]

PAM: protocerebral anterior medial, VUM: ventral unpaired medial, PPM3: protocerebral posterior medial 3, NPF: neuropeptide F, DILP: *Drosophila* insulin-like peptides.

horizontal lobes [25,26]. Functionally, there exist distinct classes of PAM neurons that can impart positive (for example, the 15 MB-M8 neurons labeled in the 0279-Gal4 strain) and negative (for example, the 3 MB-M3 neurons labeled in the NP5272-Gal4 strain) valence, and they innervate distinct parts of the mushroom bodies.

Classic associative learning assays, where flies are taught to associate a stimulus (for example sugar or electric shock) with a neutral cue (usually an odor), are commonly used to assess neural coding of reward and aversion. Genetic inactivation of most PAM neurons (with R58E02-Gal4 or 0104-Gal4 transgenes that express the yeast transcriptional activator

GAL4 in specific PAM neurons to facilitate their genetic manipulation) [Table 1] blocks appetitive learning with sucrose [25,26]. Moreover, R58E02 neurons increase activity in response to sucrose ingestion, responding more strongly following food deprivation [26]. These results suggest that the PAMs encode the rewarding value of sucrose. Conversely, inactivation of the MB-M3 neurons (NP5272-Gal4) blocks aversive learning [28]. Importantly, activation of either the MB-M8 or MB-M3 neurons substitutes for the unconditioned stimulus (sugar or shock), and is sufficient for appetitive or aversive reinforcement, respectively [28,35]. The activation of both the positive and negative valence population of all 130

PAM dopaminergic neurons promotes appetitive reinforcement [25,26]. Further investigation is needed to delineate the precise profile of the broadly targeted PAM neurons. For example, the profile of the subpopulations of neurons in the PAM cluster (other than the MB-M8 and MB-M3) is still largely uncategorized and have unknown functions.

Recent findings suggest that innate behaviors critical for survival, such as seeking food or even water, may be modulated by dopaminergic neurons that are also implicated in appetitive reinforcement learning paradigms. A group of approximately 55 PAM neurons (*R48B04-Gal4*) that includes neurons that project to the $\gamma/5$ lobe is necessary and sufficient for promoting water reward memory in a thirst-dependent manner [22]. Moreover, these neurons are activated by water intake in thirsty flies. This finding indicates that water, like sucrose, may be encoded in similar reward pathways. Activity in a nonoverlapping set of β' -projecting PAM neurons (also from the *R48B04* pattern) is necessary for innate water seeking in thirsty flies, and importantly, the γ and β' -projecting dopaminergic PAM neurons are exclusively involved in thirst-dependent learned and innate water seeking, respectively [22]. These results suggest that PAM neurons involved in other positive reward-seeking may be further categorized into innate and learned subdivisions.

Paired posterior lateral 1 neurons

The 12 paired posterior lateral 1 (PPL1) dopamine neurons synapse onto areas of the mushroom body that are largely distinct from the PAMs, including the medial (MB-MP1) and vertical (MB-MV1) lobes of the mushroom bodies [29]. The PPL1 MB-MP1 neurons, like the PAM MB-M3s, are involved in negative valence assignment [16,28]. PPL1 neurons integrate the satiety state (hungry or well-fed) of the fly in the context of learning and memory; well-fed flies form appetitive associations poorly, however, inactivating MB-MP1 neurons (*c061-Gal4*) allows retrieval of appetitive memory [30]. Conversely, activation of the MB-MP1 neurons can block appetitive memory retrieval in hungry flies [30]. *In vivo* calcium imaging shows that the PPL1s are tonically active in the fed state but are greatly attenuated in the food-deprived state [23,36]. Together, these results suggest that in well-fed flies, the dopaminergic PPL1 neurons send tonic inhibitory signals to the mushroom bodies to suppress appetitive feeding behavior.

Ventral unpaired medial neurons in sensing sugar

Dopamine also tunes the sensory perception of appetitive cues. Fruit flies, like blow flies, extend their proboscis upon detection of palatable gustatory cues through taste sensilla located on the proboscis or the distal tarsal leg segment [47]. Taste reception is largely mediated by independent population of sugar-sensing and bitter-sensing gustatory receptor neurons that send axonal projections to the subesophageal ganglion (SOG) in a modality (e.g., sweet/bitter) and organ-specific (e.g., labellum/tarsal segment) arrangement [48]. A single dopaminergic neuron located in the SOG, the ventral unpaired medial neuron (TH-VUM), is necessary and sufficient to promote proboscis extension to sucrose and further, its tonic activity is increased in starved flies [31]. The TH-VUM

makes synaptic connections broadly throughout the SOG. In addition, dopamine acts directly on sugar-sensing taste neurons to enhance taste reactivity in starved flies. However, the specific dopamine neurons responsible for this sensory tuning need to be identified [32].

Larval dopamine neurons in feeding motivation

There are approximately 90 dopaminergic neurons in the 3rd instar larval central nervous system. Notably, three bilateral clusters of dopamine neurons called the DM, DL1, and DL2 project to higher brain regions in the protocerebrum including the mushroom bodies [49]. Larvae exhibit appetitive mouth hook contractions that scale with satiation state, sucrose concentration, food source accessibility (easy to eat soft vs. more difficult to eat agar-embedded food), and with exposure to food-like odors [17]. Laser ablation of DL2 neurons that project to the larval lateral protocerebrum abolishes the food-like odor enhanced mouth hook contractions and their genetic activation is sufficient to increase mouth hook contractions [17]. Moreover, food-like odors increase DL2 neuronal activity, indicating that specific dopamine neurons in larvae react to appetitive cues to promote feeding behavior.

Mushroom bodies

The activity of mushroom body Kenyon cell neurons that are postsynaptic to the PAMs and PPL1s is necessary for both appetitive and aversive conditioning, and distinct regions of the mushroom bodies have valence-specific roles [35]. Appetitive-encoding PAM neurons specifically innervate the β lobe surface and core neurons, whereas the aversive-encoding PAM neurons exclusively innervate the β surface neurons [35]. In particular, the α/β surface neurons are necessary for both appetitive and aversive conditioning, whereas the α/β core neurons are specific for appetitive conditioning [35]. In a differential aversion conditioning paradigm, flies are trained to choose between a 30 V and 60 V electric shock-conditioned odorant: the flies avoid the 60 V-paired odorant, but also actively approach the 30 V-paired odorant. In this paradigm, both the α/β core Kenyon cells and appetitive dopamine neurons are necessary for the flies to approach the less “hazardous” odor [35]. These and other experiments argue that the PAM to mushroom body appetitive neural pathway encodes positive valuation even when the positive value is simply “less bad” rather than “good.” Because aversive conditioning is impaired in starved flies, it would be interesting if the PPL1-MP1 and PPL1-MV1 also gate relative aversive conditioning, similar to appetitive conditioning in well-fed flies.

Downstream of the Kenyon cells are 34 mushroom body output neurons (MBON) comprising 21 cell types that are glutamatergic, GABAergic, or cholinergic. MBONs elaborate zonal dendritic innervation patterns along the vertical and horizontal stalks of the mushroom bodies. Interestingly, the dendrites of glutamatergic and GABAergic MBONs are largely restricted to the β , β' , and γ horizontal regions, whereas cholinergic fibers predominately occupy the α and α' vertical stalks. The dendrites of select MBONs and presynaptic terminals of PAM and PPL1 dopaminergic neurons overlap, likely forming relays at the mushroom bodies [50,51]. Some MBONs

elaborate presynaptic endings in close proximity to dopamine neuron dendrites, implying that the MBONs may form a feedback loop to modify the dopamine to mushroom body circuit.

A recent study methodically characterized the role of each MBON cell type for a spectrum of behaviors, including both innate and learned appetitive and aversive responses [51]. Inactivation of specific glutamatergic MBONs that innervate the tips of the β and γ lobe impair appetitive and aversive conditioning [50,51]. The requirement for activity of specific cholinergic neurons varies with the appetitive conditioning paradigm being tested [50]. Interestingly, some of the same glutamatergic MBONs (β 2 and γ 5 innervating) display decreased or increased activity when exposed to odors previously paired with a reward or punishment, respectively [50]. In the context of innate behavior, remote activation of the β and γ lobe tip MBONs with the red-shifted channelrhodopsin Chrimson, promotes innate avoidance of red light [51]. Intriguingly, blocking the output of the same MBONs changes naïve odor avoidance to attraction [50]. Current models argue that the MBONs bias selection of behavioral actions and this selection bias is modified by appetitive or aversive associations [50,51].

Dopamine and drugs of abuse

Behavioral responses to drugs of abuse that can be easily measured in model organisms can be categorized as unconditioned and conditioned. Unconditioned behaviors include drug sensitivity, attraction or aversion, and locomotor effects such as hyperactivity and stereotypies. Conditioned behaviors arising from prolonged or repeated drug intake include the development of drug tolerance and sensitization, preference, withdrawal, and reinstatement following a period of abstinence. As with feeding behaviors, these responses are complex and are likely coded by multiple neural circuits acting simultaneously. The drugs of abuse that are most well-studied in *Drosophila*, ethanol and cocaine, elicit behavioral responses that remarkably parallel those in vertebrates. For example, ethanol stimulates locomotion at low doses and causes incoordination and sedation at higher doses [9]. Flies also show dose-dependent attraction and aversion to ethanol. Flies develop a preference for ethanol, find it rewarding, show signs of withdrawal, and reinstate intake following a period of abstinence.

Drug sensitivity and tolerance

A role for dopamine in the acute sensitivity to drugs of abuse was first described using pharmacological and genetic techniques that affect all or many dopamine neurons simultaneously. Cocaine binds to the plasma membrane dopamine transporter, blocking dopamine reuptake following its release at synapses, resulting in higher and more sustained extracellular dopamine. Volatilized (crack) cocaine provided at moderate doses increases locomotor activity (hyperactivity) and causes stereotypies, or repeated motor behavioral patterns [52–54]. Moderate doses of ethanol and nicotine also cause hyperactivity, and larvae fed amphetamine shows

dopamine-dependent hyperactivity [55]. Adult flies made dopamine deficient become resistant to the acute behavioral effects of ethanol, cocaine, and nicotine, suggesting that dopamine is a common target for drugs of abuse in flies, as it is in mammals [52].

The dopaminergic step of the circuitry for acute ethanol promotion of hyperactivity is known. Genetic inactivation of either most or even just a pair of dopamine neurons decreases ethanol-induced hyperactivity, whereas selective acute activation of the same pair of dopamine neurons promotes hyperactivity [14]. The pair of neurons is in the protocerebral posterior medial 3 (PPM3) cluster of dopamine neurons, and they make presynaptic contact with a circular structure termed the ellipsoid body that is part of the central complex in the fly brain. Moreover, postsynaptic D1-like dopamine receptors (DopR1) located in the ellipsoid body intrinsic neurons promote ethanol-induced hyperactivity. The central complex is a group of four highly interconnected brain structures that appears to integrate sensory and internal states to coordinate behavioral responses, including locomotion [56].

Circadian control of arousal state involves dopamine and is affected by methamphetamine and cocaine. Arousal is heightened in the daytime (except when flies partake in a midday “nap”) and suppressed in the nighttime [57,58]. Dopamine promotes wakefulness: dopamine deficient flies sleep more and flies genetically manipulated to acutely activate dopamine neurons sleep less [15,59,60]. Methamphetamine, which binds to dopamine and other monoamine transporters and results in higher extracellular dopamine levels, decreases nighttime sleep [59]. Similarly, cocaine, when provided in the flies food, decreases sleep and increases arousal state [61]. Cocaine heightened arousal works through the D1-like dopamine receptor DopR1: flies lacking DopR1 show increased nighttime sleep, and are resistant to cocaine. A second form of arousal is induced by repeated environmental stress and is also dopamine-dependent and affected by cocaine [61]. The wake-promoting effects of methamphetamine, like cocaine, depends on the DopR1 receptor, and interestingly, this function localizes in part to the mushroom bodies [62]. Consistent with this, methamphetamine, as well as exogenously supplied dopamine, restores a form of mushroom body-dependent aversive learning that is compromised by sleep deprivation [63]. Finally, we note that ethanol sedation sensitivity varies with circadian time (and so likely with arousal state), and circadian genes regulate ethanol sedation tolerance; however, the role of dopamine in circadian regulation of these and other ethanol responses is not yet known [64,65].

Dopamine neurons that project to a region of the central complex called the fan-shaped body promote wakefulness [15,60]. Further, DopR1 functions in the ellipsoid body for stress-induced arousal [61]. Taken together with the dopaminergic promotion of ethanol-induced hyperactivity mapping to the ellipsoid body, it is possible that the highly interconnected central complex is a site of motor control connected to different forms of behavioral arousal [14].

Drug preference and reward

Dopamine is also critical for more complex ethanol-related behaviors, including a form of ethanol preference and also

ethanol reward. Female flies given a choice between food with and without added ethanol will lay their eggs on the ethanol food: ethanol is present in decomposing fruit, the preferred food source and gathering place for *Drosophila* in the wild [21]. Dopamine neurons in the PAM and PPM3 (the same neurons that promote ethanol hyperactivity) clusters promote egg-laying preference, whereas dopamine neurons in the PPL1 cluster inhibit egg-laying preference. Importantly, blocking neuronal activity in the PAMs labeled by *R58E02-Gal4* is ineffective, distinguishing egg-laying preference for ethanol food from appetitive learning with sucrose [25,26]. Both the PAM and PPL1 neurons tested are presynaptic to the mushroom bodies, and genetic inactivation experiments show that the α/β' mushroom body neuropil promotes an egg-laying preference for ethanol food.

Ethanol is rewarding to *Drosophila*. The presence of reward is shown by a positive association between ethanol intoxication and co-presentation of a neutral odor cue: the neutral cue becomes attractive when later presented alone [27]. Importantly, flies perform work (they tolerate an aversive electric shock) to approach the previously ethanol-paired odor. Blocking either dopamine synthesis or dopamine synaptic transmission completely disrupts this ethanol conditioned preference. Blocking dopamine synaptic transmission is, perhaps surprisingly, not effective during either the pairing or consolidation phase as one might expect from mammals, but is effective when the fly is asked to remember the odor-ethanol intoxication pairing [66]. The lack of effect during learning about ethanol reward may be due in part, however, to tools used: all but the PAM neurons were inactivated. Finally, we note that the appetitive valuation of ethanol is evident only after an initial period of conditioned aversion, highlighting the complexity of behavioral encoding for this and other addictive drugs [27].

Drug targets downstream of dopamine

The mushroom bodies and the central complex, innervated by dopaminergic and other types of neurons, are critical for a broad spectrum of ethanol and other drug-related behaviors. Our understanding of the role of the central complex in ethanol behaviors is still rudimentary, however, specific classes of ellipsoid body neurons are important for ethanol-induced hyperactivity and ethanol sedation tolerance [14,67,68]. The mushroom bodies promote ethanol-induced hyperactivity, ethanol preference, ethanol reward, and recovery from sedation induced by the related benzyl alcohol [21,27,67,69,70]. Functional mapping of the mushroom body for ethanol behaviors, while still preliminary, suggests the use of specific neuropils for simpler behaviors, and sequential use of distinct neuropils for more complex behaviors. For example, sequential use of the γ , α/β' , and α/β neuropils supports acquisition, consolidation, and retrieval of ethanol reward memory, respectively [27].

The circuitry for ethanol behaviors extends to both cholinergic and glutamatergic MBONs [51]. Intriguingly, the dendritic arborization patterns of ethanol reward and aversion MBONs largely overlap with the presynaptic terminals of PAM and PPL1 dopaminergic clusters [50,51]. For example, activity in the cholinergic MBON- $\alpha/2$ is required for the

expression of the appetitive response to alcohol conditioned odorants [51]. The PPL1s are currently the only known MB extrinsic neurons to project into the $\alpha/2$ region of the vertical lobe, suggesting that the PPL1 to MBON- $\alpha/2$ circuitry is critical for alcohol reward learning [27,71].

Dopamine: food and drugs

Distinct, valence-specific dopaminergic neurons that target the mushroom bodies seem to be engaged by the rewarding properties of both food and drugs.

Dopamine neurons

The PAM cluster of dopamine neurons is necessary for ethanol preference, appetitive reinforcement, aversive reinforcement, and water attraction and reinforcement [21,22,25,26,28]. Importantly, nonoverlapping sets of PAM neurons are critical for innate water attraction and water reinforcement, utilizing distinct yet anatomically related dopaminergic pathways. Similarly, a group of approximately 40 PAM neurons that is distinct from PAM sucrose and water reinforcement neurons is critical for ethanol egg-laying preference [21]. These results indicate that the PAM cluster is a heterogeneous mixture of neurons that can drive both innate and learned behaviors. However, the valence-specific role of PAM subsets in the study of drugs is still unclear.

Investigations directly targeting the PPL1-MP1 neurons implicate their function in assigning negative valence (odor-shock), and they are also necessary for ethanol aversion [21,28,30]. These results suggest that stimuli with an aversive property such as electric shock and ethanol may converge onto a common dopaminergic pathway. Because an unconditioned stimulus like ethanol has simultaneous rewarding and aversive properties, it is possible that its behavioral actions are encoded by both the aversive (ex. PPL1-MP1, MV1; PAM-M3) and reward (ex. PAM-M8) circuits. Complex stimuli with both rewarding and aversive properties may be processed in parallel by separate valence-specific dopamine circuits. Interestingly, the PPL1 cluster may also code for appetitive functions: because the PPL1 to MBON- $\alpha/2$ terminal endings and dendrites form a putative circuit, it is possible that PPL1 activity facilitates the transition from aversive to appetitive alcohol reward [27,71]. Specific manipulation of PPL1 and other dopaminergic clusters is needed to verify the neuronal substrate mediating the appetitive alcohol response.

The locomotor stimulant effects of ethanol and innate ethanol preference are localized in part to the PPM3 dopamine cluster [14,21]. Similarly, the wake-promoting effects of dopamine utilize PPM3 neurons [15]. However, there is, as yet, no reported role of the PPM3s in food-associated behaviors. The PPM3 pathway may code for aspects of arousal or attention that underlie specific forms of motivated behavior [72].

Dopamine neuron postsynaptic targets

Ethanol reward converges on some of the same pathways as sucrose reward because the γ , α/β' , and α/β mushroom body neurons are involved in similar phases of appetitive memory

acquisition, retrieval, and consolidation [27,37]. Perhaps even more compelling is the remarkably similar set of MBONs required for 2 h odor-sugar appetitive memory and odor-ethanol intoxication memory. Both forms of appetitive association require neuronal activity in the same glutamatergic MBONs innervating the β and γ lobes, and also the same cholinergic MBONs innervating the α and α' lobes [51]. Therefore, the reward circuits for appetitive conditioning of odors for sugar and ethanol converge at or just beyond the mushroom bodies.

Tyramine and octopamine

Tyramine and octopamine, often called the trace amines, are synthesized in sequential steps from L-tyrosine. Tyrosine is converted into tyramine by tyrosine decarboxylase, which is encoded by functionally interchangeable products of the *Tdc1* and *Tdc2* genes; *Tdc2* encodes the major neuronal form. Tyramine is converted into octopamine by tyramine β hydroxylase, encoded by the *T β h* gene. *Tdc2* mutations reduce levels of both tyramine and octopamine, whereas *T β h* mutations reduce levels of octopamine but also increase tyramine by about 10-fold [73]. This interrelationship can complicate the assignment of a particular trace amine to behavioral functions. T β h (and so octopamine) is present in about 150 cells in the adult brain [74]. Surprisingly little is known about the numbers and innervation patterns of tyraminer-gic cells. Similarly, the role of individual octopaminergic neurons, their innervation patterns, and their connectivity are just beginning to be explored. There are two classes of octopamine receptors in flies, including one α -adrenergic-like (OAMB) and three β -adrenergic-like (Oct β 1R, Oct β 2R, and Oct β 3R), and three tyramine receptors [75,76]. The trace amines tyramine and octopamine likely bestow vertebrate epinephrine and norepinephrine functions, respectively.

Tyramine and octopamine in feeding behavior

Classic experiments in honey bees show that electrical stimulation of a single octopaminergic neuron or direct administration of octopamine to the olfactory antennal lobe or the mushroom bodies supplants the rewarding properties of sucrose in odorant conditioning assays [77,78]. This led to the identification of the octopaminergic ventral unpaired median neuron 1 of the maxillary neuromere (VUMmx1) as the neuron that conveys the rewarding value of sucrose. It is located beneath the SOG (the first gustatory relay site), and it has dense ramifications onto the antennal lobe, lateral protocerebrum, and mushroom bodies [77].

Octopamine is necessary for innate and learned appetitive behaviors

Similar to the honey bee VUMmx1, in *Drosophila*, there are three clusters containing 8–10 octopaminergic VUMs each that have widespread arborizations in the deutocerebrum and protocerebrum, including in the latter the antennal lobe and mushroom bodies [79]. *T β h* mutant adults are unable to form

short-term sucrose reward memories or extend their proboscis in response to tarsal stimulation with sucrose; both behavioral deficits can be rescued by feeding T β h-deficient flies octopamine [34,38,80]. Normal sucrose responsiveness to tarsal stimulation is restored in T β h-deficient flies by expression of T β h in 34 SOG and 11 antennal lobe neurons (labeled by NP7088-Gal4) [38,74].

Octopamine is upstream of protocerebral anterior medial neurons for learned behaviors

Recent work using appetitive conditioning tests confirms that T β h-deficient flies are unable to form appetitive memories; however, appetitive memory can be acquired in flies that are T β h-deficient when ~90 PAM dopamine neurons (*R58E02-Gal4*) are acutely activated [26]. This result suggests that octopamine signaling may act upstream of or in parallel with the PAM neurons. Similarly, expression of an RNAi directed against the OAMB octopamine receptor in ~40 PAM neurons (*0104-Gal4*) blocks appetitive conditioning with the sweet but non-caloric sugar arabinose, and brain application of octopamine increases the activity of these same neurons [25]. Another group showed that OAMB is strongly and selectively expressed in the α/β mushroom body lobes where it promotes appetitive conditioning [76].

Octopamine encoding of sweet palatability

Blocking the output of a subset of octopaminergic and tyraminer-gic neurons (labeled by *Tdc2-Gal4*) impairs appetitive learning with arabinose, but not with sucrose (both sweet and caloric) [25]. Two other independent studies show that flies exhibit enhanced appetitive reinforcement when an exclusively sweet sugar is supplemented with exclusively caloric sugars [81,82]. Together, the results suggest that sucrose has two independent reinforcing properties, its sweet and nutritive value. Importantly, octopamine most likely encodes sweet palatability, whereas a caloric sensor, perhaps also octopaminergic, is responsible for memory reinforcement that remains to be identified. It is important to note that appetitive conditioning by direct activation of octopamine neurons is short-lived (reported to last for 30 min) compared to sucrose conditioning, and it does not depend on the satiation-state of the fly [25].

Octopamine in larval feeding behavior

Octopamine also promotes appetitive behavior in larvae. T β h-deficient larvae have diminished, starvation-induced mouth hook contractions that can be rescued by feeding the larvae octopamine [39]. Moreover, inactivation or activation of *Tdc2-Gal4* neurons showed that these neurons are necessary and sufficient to promote this appetitive response. Targeted laser ablations indicate that larval octopaminergic VUM1 and VUM2 neurons inhibit and promote the larval feeding response, respectively. Interestingly, the motivated feeding behavior is only observed when larvae are provided with soft liquid food as compared to agar-embedded sugar. This could mean that an aversive condition that requires work (extra energy

expenditure) may prevent the expression of octopamine-dependent appetitive behaviors.

Tyramine and octopamine and drugs of abuse

Ethanol sensitivity, ethanol tolerance, and ethanol preference are all regulated by the trace amines. Ethanol sedation sensitivity is decreased when synaptic output is blocked in a subset of Tdc2 neurons (*Tdc2-Gal4*), and feeding of tyramine but not octopamine to these synaptically silenced flies restores ethanol sensitivity, implicating tyramine in the sedative effects of ethanol [83]. While ethanol sensitivity is unaffected in *Tβh* mutants, the development of ethanol tolerance is compromised, raising the possibility that initial sensitivity is tyramine-dependent, and neuroadaptation to repeated exposures is octopamine-dependent [84,85]. Similarly, sensitivity to acute crack cocaine is increased in *Tdc2* mutants and when *Tdc2-Gal4* neurons are hyperpolarized but is unaffected in *Tβh* mutants, suggesting that similar to ethanol, tyramine regulates cocaine sensitivity [86].

Flies are attracted to the smell of ethanol at low concentrations when presented alone or mixed with food. This innate olfactory preference can be measured by trapping flies that come in proximity to the odor source [40]. Innate olfactory preference for ethanol is lost in *Tβh* mutants and is regained when Tβh activity is restored to a small number of Tβh neurons that are likely to release acetylcholine in addition to the trace amines. While the individual neurons responsible for ethanol olfactory preference remain to be identified, the implicated cells are located in the subesophageal region of the fly brain.

Tyramine and octopamine: food and drugs

In the context of drugs, Tβh-deficient flies' sensitivity is unaltered upon exposure to alcohol or cocaine, which implies that sensitivity may be mediated by tyramine signaling. Moreover, octopamine activity may be important in mediating ethanol tolerance. Currently, there are no known feeding behaviors that are associated with tyramine.

Octopamine promotes innate and learned behaviors

The pioneering work in honey bees has implicated that octopamine is necessary for the rewarding value of sucrose [77]. Indeed, the notion that octopamine is an important transmitter of sucrose reward is consistent in flies. Two independent studies have shown that Tβh mutant flies are unable to form sucrose-reinforced memory or exhibit normal PER in response to sucrose stimulation [34,38]. These results suggest that octopamine is necessary for appetitive conditioning and innate responses to sucrose.

Interestingly, the ventrally located OA-VUMs are implicated in mediating innate alcohol approach and sucrose-induced PER [38,40]. Octopaminergic neurons that are responsible for appetitive learning for sucrose are attributed to the VUM-a6, a7, a8, and VPM 3 (labeled in NP7088) [25]. Thus, while direct overlap has not yet been proven, the OA-

VUMs may have multiple modulatory roles in alcohol olfactory approach, gustatory sugar sensitivity, and appetitive conditioning.

Octopamine encodes appetitive value

Interestingly, it has been shown recently that blocking *Tdc2-Gal4* neuronal activity prevents appetitive short-term memory acquisition with arabinose but not sucrose, and this is because it is both sweet and caloric [25]. Compared with the earlier studies investigating the role of octopamine in learning, Tβh-deficient flies could not make appetitive associations with sucrose reinforcement [34]. It is important to consider that not all octopaminergic neurons in the *Drosophila* brain are labeled in the *Tdc2-Gal4* pattern; thus, it may be possible that other octopaminergic neurons are mediating the calorie-dependent appetitive conditioning. Alternatively, Tβh-deficient flies also show reduced PER for sucrose, whereas silenced *Tdc2-Gal4* flies are unaffected [31,38]. Another study shows that Tβh-deficient flies are able to form water reinforced memories in a novel water-reward learning paradigm, which is consistent with the model that octopamine encodes the sweet palatability of sugars [22]. Taken together, these results suggest that depending on the internal motivational context (hunger, thirst, satiety), sweetness, nutritional content, and even water is rewarding to flies and may be ultimately encoded through dopaminergic reward pathways. Defining the role of octopamine in drug preference and reward in concert with refining the dopaminergic circuitry will be important for developing comparative circuit-based models of appetitive processes in feeding and addiction.

Neuropeptide F

Drosophila expresses NPF, which is related evolutionarily to mammalian NPY, and the separately encoded short neuropeptide F (sNPF) that shares a RxRF C-terminal motif with NPF. NPF is present in only 20–26 neurons in the adult brain (10–13/hemisphere), whereas sNPF is expressed in approximately 280 neurons in the brain and most or all mushroom body Kenyon cells [87]. NPF and sNPF are co-expressed in four neurons. Similar to dopamine, NPF is implicated in a variety of motivated behaviors like learning and memory, feeding, drug seeking, and odorant attraction [4,30,41,42]. sNPF regulates bitter taste responsiveness and also larval food intake, but its role in drug-related behaviors is not known [43,88].

Neuropeptide F in feeding behavior

Larval feeding behavior

Neuropeptide F acts upstream of dopamine to promote appetitive behavior in larvae. The single neuropeptide F receptor (NPFR) is expressed in many dopaminergic neurons in larvae, including DL2 neurons, and RNAi against NPFR in dopamine neurons blocks both appetitive odor enhancement of DL2 neuronal activity and feeding behavior [17]. Furthermore, silencing NPF neurons not only blocks this appetitive

odor enhanced feeding, but also in starved larvae decreases feeding behavior on solid (unpalatable) but not liquid (palatable) food [39,44]. NPF neuron silencing also decreases food intake on quinine-adulterated food [4]. Because NPF neuronal activity manipulation does not affect appetitive behaviors on more palatable food sources, the NPF system may be critical in situations that require risky behavior with aversive conditions [4,39,89].

Adult feeding behavior

Enhancement of NPF neuronal signaling increases food intake in food-deprived adult flies, and it also increases sugar but not bitter taste reactivity when tested in fed adult flies [31,45]. NPF-enhancement of sugar taste reactivity is blocked in DopEcR-deficient flies, suggesting that NPF signaling is upstream of or in parallel with dopaminergic neurons that modulate sugar sensitivity. Importantly, the same manipulation of NPF neuron activity does not enhance tolerance of a bitter compound (lobeline) mixed with sucrose [43]. These results suggest that NPF may not promote innate appetitive behavior under aversive conditions in the adult fly, and this distinction from NPF's role in larvae may be important in determining the shift from continuous feeding in larvae to selective feeding in adults.

Well-fed flies are much less able to form appetitive memories [90]. However, activation of NPF neurons during retrieval in well-fed flies allows the expression of a previously formed appetitive memory, indicating not only that appetitive memories are well-formed but suppressed in fed flies, but also suggesting that NPF activity mimics the state of hunger [30]. Consistent with this notion, reduced expression of NPFR in the aversive-encoding PPL1-MP1 dopaminergic neurons blocks appetitive memory formation in hungry flies [30]. This evidence suggests that NPF signaling is upstream of PPL1 neurons, perhaps keeping them turned "off" in hungry flies to promote appetitive behaviors.

Neuropeptide F also promotes innate attraction to appetitive odors in food-deprived flies [41]. Inhibition of NPF neurons decreases food odor attraction in starved flies, and conversely, activation of NPF neurons promotes robust food odor attraction in fed flies. The activity of four NPF neurons in the dorsal protocerebrum is highly correlated with food odor attractiveness [41]. Intriguingly, NPF neuron activation in response to fruity odorants was high even in satiated flies, corresponding to robust behavioral attraction. Collectively, the evidence supports the role of NPF as a molecular signature encoding the motivational state of the fly. NPF activity functions in innate and conditioned contexts and signals upstream of dopaminergic (and likely other) neurons to mediate satiation-state dependent behaviors such as sugar taste reactivity and memory expression.

Neuropeptide F and drugs of abuse

Neuropeptide F regulates acute ethanol sensitivity, ethanol preference, and ethanol reward. Ethanol sedation sensitivity is reduced when NPF expressing cells are either ablated or synaptically silenced specifically during ethanol exposure [91].

Conversely, NPF overexpression in NPF neurons increases ethanol sedation sensitivity. Interestingly, NPF expression is increased following exposure to intoxicating levels of ethanol [42]. Thus, NPF signaling actively promotes sensitivity to ethanol intoxication.

Mating history and the presence of predators also regulate NPF expression. NPF levels are lower in sexually rejected males, higher in mated males, and rejected males show an increased preference for ethanol [42]. Blocking NPF signaling by genetically reducing NPFR levels increases ethanol preference in mated males and conversely, acute activation of NPF neurons in inexperienced males blocks ethanol preference. Importantly, both activation of NPF neurons and mating are rewarding to the fly since neutral odors paired with either manipulation become attractive when later presented alone. Finally, artificial activation of NPF neurons interferes with the ability of flies to find ethanol rewarding. Adult flies cocultured with natural predator wasps lay more eggs on food containing ethanol concentrations (15%) that are toxic to the predators [92]. The visual presence of predators decreases NPF expression in the fan-shaped body region of the brain, and transgenic increases in NPF block the predator-driven egg-laying preference for ethanol. Taken together, these findings are consistent with NPF responding to rewarding and threatening stimuli to set the valuation of drug reward. It is not yet known if the role of NPF in ethanol sensitivity, reward, and preference are anatomically linked.

Neuropeptide F: food and drugs

Neural targets of neuropeptide F

Neuropeptide F is an upstream modulator of satiation-state dependent behaviors such as odorant-enhancement of larval mouth hook contractions, appetitive reinforcement, retrieval of appetitive memory, innate olfactory attraction, sugar sensitivity, and motivated feeding in larvae and adult flies [4,17,30,41–43,45]. Interestingly, the currently known downstream targets of NPF are dopaminergic neurons. For example, in larvae, appetitive odorant-induced mouth hook contractions require NPF signaling into DL2 neurons. In adult flies, NPF disinhibits the PPL1 neurons to allow starvation-dependent memory retrieval. More recently discovered in adult flies, NPF promotes sugar sensitivity and may be upstream of the TH-VUM neuron located in the SOG [31,43].

Food and drug similarities in learning and memory

The activation of NPF neurons is sufficient for appetitive conditioning, similar to the functional role of the dopaminergic PAM cluster [42]. In the context of alcohol preference, activation of NPF neurons during ethanol conditioning impairs 24 h appetitive memory, but an immediate aversive memory (within 30 min posttraining) that is formed during the same conditioning is intact [27]. Thus, transient NPF activity during the paired ethanol odorant phase seems to block the late stage ethanol attraction. In contrast, dopaminergic activity in neurons (labeled by *TH-Gal4*) is necessary only during the retrieval phase [27]. These two pieces of evidence

suggest that NPF and distinct dopaminergic clusters must coordinate neural activity at particular phases of learning for proper expression of alcohol-conditioned appetitive memory. Moreover, the exact neuronal substrates that may be encoding the rewarding aspects of ethanol preference is still unknown.

Neuropeptide F encodes hunger, reward status, and innate attraction

In *Drosophila*, NPF has three distinct putative functions in hunger, reward status, and innate attraction. It is unclear if the entire NPF circuitry coordinates each of the putative functions in a motivational context-dependent manner or if distinct NPF neurons assign value similar to the valence-specific dopaminergic circuitry. Moreover, the involvement of NPF with other drugs of abuse such as cocaine, nicotine, and amphetamines has yet to be explored.

Drosophila insulin-like peptides

In *Drosophila*, there are eight insulin-like peptides and one insulin receptor (dInR) [93,94]. Here, we limit our discussion to the direct functions of the brain-derived DILPs expressed within the median neurosecretory cells of the pars intercerebralis, DILP2, 3, and 5, and their effects on feeding and drug behaviors [95]. The central neural mechanisms and systemic neurohemal modulators that may control the local secretion of DILPs into the central nervous system are covered extensively in other reviews [95].

Drosophila insulin in feeding behavior

3rd instar larval behavior

Shen et al. showed that pan-neuronal misexpression of DILP2 significantly decreases larval mouth hook contractions on both unpalatable (solid or quinine-adulterated) and palatable (liquid) food [4,44]. Importantly, expression of a dominant negative dInR in NPFR neurons increases mouth hook contractions in fed larvae, whereas expression of a constitutively active form of dInR in NPFR neurons significantly attenuates mouth hook contractions in starved larvae [4,44]. These findings highlight that insulin is a potent modulator of feeding that can negatively regulate neurons downstream of NPF. Interestingly, manipulation of dInR activity in NPFR neurons only affected feeding on unpalatable substrates, however, overexpression of DILP2 negatively regulated mouth hook contractions on both palatable and aversive substrates, suggesting the existence of NPFR-independent pathway for insulin in palatable feeding [44].

Adult fly behavior

In capillary feeding preference assays, well-fed flies exhibit an initial preference for highly palatable sugars over less palatable yet more nutritious sugars. However, over time, there is a clear shift in preference toward substrates with greater caloric content [46]. Therefore, adult flies have a preference for caloric

sugar in a starvation-dependent manner. Well-fed DILP2 or DILP3-deficient adult flies prefer to consume a less palatable but caloric mixture of sucrose and mannose (1:4 ratio) versus the sweet but noncaloric L-fucose [95]. Because these mutants behave like starved flies, the results imply that DILP2 and DILP3 encode a state of satiety. Perhaps surprisingly, then, genetically silencing DILP3 cells does not increase the probability of proboscis extension in response to sucrose [31]. Moreover, activation or inactivation of DILP2 and DILP3 cells does not affect water consumption [96]. Finally, the transient activation of DILP2 cells during appetitive memory retrieval does not block approach to a sucrose-conditioned odorant [97]. All together, these results suggest that insulin activity may influence palatable versus nutritional food preference instead of satiety state.

Insulin and drugs of abuse

Insulin signaling is implicated in both adult ethanol sensitivity and in the long-term physiological effects of developmental ethanol exposure. A 50% reduction in dInR expression increases ethanol sensitivity without affecting other insulin-dependent processes, including nutrient signaling and organismal growth [98]. Prolonged ethanol exposure during development does regulate these processes: flies raised on food with added ethanol are smaller and slower to develop, and show significantly suppressed cellular proliferation, concomitant with reduced expression of DILP2 and dInR in the brain [99]. The effects of developmental ethanol exposure can be reversed by overexpression of DILP2, indicating that ethanol-induced decreases in insulin signaling mediate the developmental effects of ethanol exposure.

Insulin-like peptides: food and drugs

Together the evidence in larvae and adult flies supports the notion that insulin encodes a state of repletion by negatively regulating potential targets such as NPFR neurons. Note that insulin is also a critical regulator of carbohydrate levels in the hemolymph. Thus, manipulation of DILPs may mask direct or indirect effects of neuronal substrates sensitive to nutrients [100–102]. Studies of the effects of insulin manipulation on drug-related behavior are limited to alcohol. As mentioned in the NPF section, increased NPF activity is correlated with increased ethanol sedation. Insulin could potentially function upstream of NPF to influence alcohol sensitivity. Since insulin decreases feeding whereas NPF increases feeding, it will be interesting to determine if a singular neural pathway underlies both behaviors.

Corazonin in feeding behavior and drugs of abuse

Corazonin is a neuropeptide that may be related to the mammalian gonadotropin-releasing hormone. While less is known about corazonin, a couple of recent studies indicates that neuronal corazonin regulates behavior. Hergarden et al.

showed that the activation of corazonin neurons, like NPF neurons, increases food intake in food-deprived flies [45]. Flies lacking neuronally-expressed corazonin or the cells expressing corazonin are resistant to ethanol sedation [103]. Corazonin promotes sedation sensitivity specifically in adult flies, and activation of corazonin-expressing cells increases sedation sensitivity whereas synaptic silencing decreases it. Therefore, corazonin signaling is engaged by ethanol exposure to regulate sedation sensitivity. Interestingly, the corazonin expressing cells implicated in ethanol sedation sensitivity likely project to the pars intercerebralis neuroendocrine organ that expresses the DILPs and other peptides, and they also express Gr43a, a gustatory receptor that senses internal fructose levels and regulates feeding [104,105]. Deletion of corazonin expressing cells or its receptor also causes a marked delay in recovery from sedation induced by pure ethanol [106]. Interestingly, these manipulations of corazonin signaling also decrease the activity of acetaldehyde dehydrogenase (ALDH), an enzyme critical for ethanol metabolism. Ethanol is converted into acetaldehyde by alcohol dehydrogenase, and then into acetate by ALDH. Acetaldehyde accumulation in humans is likely the cause of many of the unpleasant and toxic effects of alcohol consumption, and this work in flies seems to tie neuroendocrine signaling to the regulation of metabolism.

Perspective

In this review, we gather evidence for the behavioral actions of a limited set of neuromodulators in both feeding behaviors and drug-related behaviors. The overlap of molecules and neural substrates allows us to speculate that shared circuitry imparts shared functionality, as is similarly proposed in mammals. However, there remain important unanswered questions that preclude detailed analysis of each neuromodulator and their relationships that is critical to assign precise function. For most of the neuromodulators discussed, single cell resolution has not yet been achieved. One exception is in the fruit fly dopamine system, where there is a precedent for individual cells imparting specific functions. For example, ethanol-stimulated locomotion and the promotion of wakefulness map to specific PPM3 dopamine neurons. In another example, the tonic activity of three MB-MP1 neurons in the PPL1 cluster dictates the satiation state-dependent expression of appetitive behavior. Furthermore, because this type of comparative neuroanatomical/functional dissection of behavior is only recently possible, similar cellular resolution experiments between feeding and drug-related behaviors await future experimentation.

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Conflict of interest

None declared.

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