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Reward from bugs to bipeds: a comparative approach to understanding how reward circuits function

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ABSTRACT

In a complex environment, animals learn from their responses to stimuli and events. Appropriate response to reward and punishment can promote survival, reproduction and increase evolutionary fitness. Interestingly, the neural processes underlying these responses are remarkably similar across phyla. In all species, dopamine is central to encoding reward and directing motivated behaviors, however, a comprehensive understanding of how circuits encode reward and direct motivated behaviors is still lacking. In part, this is a result of the sheer diversity of neurons, the heterogeneity of their responses and the complexity of neural circuits within which they are found. We argue that general features of reward circuitry are common across model organisms, and thus principles learned from invertebrate model organisms can inform research across species. In particular, we discuss circuit motifs that appear to be functionally equivalent from flies to primates. We argue that a comparative approach to studying and understanding reward circuit function provides a more comprehensive understanding of reward circuitry, and informs disorders that affect the brain's reward circuitry.

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Introduction

Our bodies are innately wired to seek and respond to reward. The ability to perceive, interpret, and respond to reward is critical for the survival of an animal in its natural environment. Reward motivates animals to seek water and food, to mate, and to nurture progeny. Not surprisingly, across animals, the basic neural processes mediating rewarding responses are remarkably similar. These similarities provide a valuable opportunity to extract relevant principles underlying reward and motivated behaviors.

Studying complex behavior and its motivation requires an understanding of the genetic identity of individual neurons, their unique response profile and how they come together to form functional neural networks. In this review, we argue that this is best achieved by cross-species comparison. Many behavioral disorders, including drug abuse, addiction, depression, and anxiety, act on reward neural circuitry. A more in-depth analysis of how reward circuits function and how they are changed is therefore essential for understanding these disorders. Comparing across model systems from invertebrates to mammals is a powerful approach because it provides multiple levels of analysis of reward mechanisms: from molecules to neural systems and behavior.

Recent technological advances have made invertebrate research models particularly attractive because of the accessibility and variety of genetic tools available that afford the manipulation of genetically identified neurons with unprecedented spatial resolution. Further, although the numbers of neurons that comprise invertebrate nervous system are reduced, their neural circuitry and behavior appears remarkably complex. Together, model systems such as the withdrawal circuit of the sea slug *Aplysia californica*, stomatogastic ganglion of the crab *Cancer borealis* (30 neurons) and nervous systems of the nematode *Caenorhabditis elegans* (302 neurons), and fruit fly *Drosophila melanogaster* (100,000 neurons) have emerged as substantial contributors to our understanding of neural circuitry mechanisms.

In this review, we describe functional similarities and differences in reward circuits that are shared across model organisms. Specifically, we discuss circuit motifs, or connections between different neuronal types, that create a context within which neurotransmitters act. The goal of this review is not to provide an exhaustive comparison of all possible functionally comparable circuits for reward and motivated behavior, but to provide pointed examples that outline similarity in circuit motifs across model organisms and identify gaps in our knowledge. We concentrate specifically on dopaminergic circuit motifs because of its highly conserved role in modulating motivated behavior and reward processing. Much of this discussion focuses on the *Drosophila* and mouse models largely because the genetic tools available in these animals allow for precise spatial manipulation of reward circuitry components. We aim to emphasize the importance of better integration of research across species, through which, a comprehensive understanding of motivated behaviors can be achieved.

Functionally similar features across species

It is enticing to reason that because a behavior is important for survival in many organisms, the circuits underlying these behaviors might also share important similarities. However,

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there are many ways to organize a simple circuit, never mind an entire brain, and it is imperative not to presume that behavior similarity automatically assumes circuit similarity. Most likely, insects lack homologs of forebrain structures involved in reward processing such as the nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala (AMYG), or hippocampus. However, insects have evolved structures such as the mushroom body and central complex, which show many functional and anatomical similarities with mammalian structures that mediate reward and motivated related behaviors (Farris, 2011; Strausfeld, 2009; Strausfeld & Hirth, 2013; Wolff & Strausfeld, 2015).

We propose that despite not being able to directly compare brain structures, comparing simple circuit motifs and connectivity patterns can be informative for understanding motivated behaviors that appear remarkably similar across species. Of course this is not to say that flies possess the same level of complexity that humans do, but instead, that the foundation of this complexity may be discrete circuits that are similar in form and function and likely repeated throughout the brain. Indeed, many anatomical features of neurons, and the connectivity between neurons, are consistent across insects and rodents.

Using technology to understand circuit complexity

Parsing apart the heterogeneity of reward circuitry that exists both within and across species has been particularly difficult. The development and refinement of neurogenetic tools that allow for controlled gene expression has revolutionized the study of neural circuits underlying behavior across all research models and affords a unique opportunity to address this. Technology that allows for *in vivo* gene manipulation is both changing the types of questions scientists are able to ask and the level of detail with which they can answer. For example, binary genetic systems such as the Cre-Lox in mice and UAS-GAL4 system in flies have added the spatial resolution necessary to manipulate gene expression in discrete subsets of cells. Consequently, much of the circuitry discussed in this review focuses on these two model organisms.

Although several binary systems are available in mice, the Cre-Lox recombination system is rapidly becoming the most widespread. The Cre-Lox system provides a sophisticated way to selectively drive expression of a gene within subpopulations of neurons to generate knockouts or conditional knockouts of endogenous genes or the expression of exogenous genes such as opsins for optogenetics (Fenno, Yizhar, & Deisseroth, 2011; Gu, Zou, & Rajewsky, 1993; reviewed in Huang & Zeng, 2013; Pupe & Wallen-Mackenzie, 2015). In this system, most commonly, Cre recombinase is driven under a specific promoter, which affords spatial specificity. Cre induces recombination at inserted loxP sites, allowing expression of the effector such as an opsin or reporter. It difficult to imagine that targeting cells using endogenous promoters associated with specific neurotransmitters does not provide enough resolution, however these very studies highlight that this level of detail is still not enough to understand the complexity of neurons and their circuitry.

The UAS-GAL4 system has been used for over 20 years in *Drosophila* to provide spatial specificity and in recent years, modifications to the system have provided exquisite spatial resolution to the level of single or pairs of neurons in the fly central brain (Brand & Perrimon, 1993). In this system, yeast transcription factor, GAL4, is driven under a specific promoter, which provides spatial specificity. GAL4 binds to an upstream activating sequence (UAS), driving expression of a specific effector such as an opsin or reporter. Further, combining multiple binary systems such as the UAS-GAL4 system, LexA-LexAop system, or Flp-FRT system provides intersectional genetic approaches that significantly decrease the number of cells within an expression pattern (for comprehensive reviews, see del Valle Rodriguez, Didiano, & Desplan, 2012; Venken, Simpson, & Bellen, 2011). Suppressing function of the driver using the transcriptional repressor Gal80 driven under a specific promoter can further narrow expression patterns. However, a modification of the UAS-GAL4 system called the Split-Gal4 system is rapidly gaining momentum for this purpose (Luan, Peabody, Vinson, & White, 2006 reviewed in Luan & White, 2007). This system uses two endogenous promoters, with partially overlapping expression patterns, for the expression of a functioning GAL4. The end result is a driver line with near single neuron specificity. In combination with thermogenetics (i.e. shibire^{ts} or dTrpA1) or optogenetics (channelrhodopsins, halorhodopsin) researchers can precisely manipulate subpopulations of neurons with unprecedented resolution (Hamada *et al.*, 2008; Inada, Kohsaka, Takasu, Matsunaga, & Nose, 2011; Kitamoto, 2002; Klapoetke *et al.*, 2014). Moreover, different binary systems, such as UAS-GAL4 and LexA-LexAop can be combined such that different neurons within the same circuit can be activated or silenced in order to understand how circuits function in behaving animals.

Functional similarities of reward systems across species

Like other complex behaviors, responses to reward require interplay between all of the brain's major transmitter and modulator systems: dopamine, noradrenaline (octopamine), serotonin, acetylcholine, glutamate, GABA, and many neuropeptides. This is true across all species in which reward has been investigated (Figure 1).

In invertebrates (namely *Drosophila*), encoding reward and directing motivated behaviors requires the coordination of multiple brain regions including the antennal lobe (AL), subesophogeal zone (SEZ), mushroom body (MB), crepine neuropil (CRE), lateral horn (LH), and superior medial protocerebrum (SMP) (Figure 1(A)). Much of what is understood about the circuitry underlying reward memory in *Drosophila* was investigated using assays for memory for an olfactory cue associated with sucrose, which explains the heavy emphasis on the olfactory circuit. Other areas of the neuropil that are less well investigated such as the CRE and SMP have projections that lead from the MB to these areas. It is very likely that many other brain structures, such as the well-studied central complex, are also associated in reward

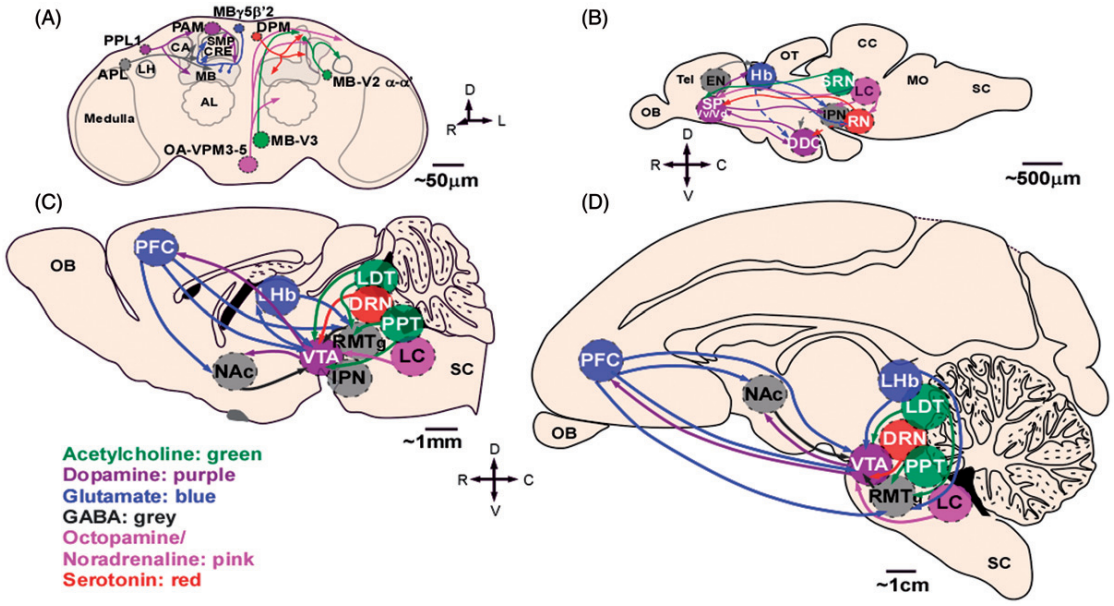

Figure 1. Reward circuitry across species: schematic of rostral view of fly brain (A) and midsaggital views of zebrafish (B), rodent (C), and primate (D) brains. For clarity only some of the projections are shown and only the main neurotransmitter is represented within each brain region (co-expression is not shown). A legend for neurotransmitter color code is included: green, acetylcholine; purple, dopamine; gray, GABA; blue, glutamate; pink, octopamine/noradrenaline; red, serotonin. Dotted lines represent presumed connectivity. Abbreviations: APL: anterior paired lateral neuron; CA: calyx; CC: cerebellum; CRE: crepine neuropil; DDC: dopaminergic diencephalic cluster; DPM: dorsal paired medial neuron; DRN: dorsal raphe nucleus; IPN: interpeduncular nucleus; LHb: lateral habenula; LH: lateral horn; LDT: laterodorsal tegmentum; LC: locus coeruleus; MB: mushroom bodies; MO: medulla oblongata; NAc: nucleus accumbens; OB: olfactory bulb; OT: optic tectum; PAM: dorsomedial anterior protocerebral; PFC: prefrontal cortex; PPT: pedunculopontine tegmentum; RN: raphe nucleus; rRC: rostral raphe complex; RMTg: rostromedial tegmental nucleus; SC: spinal cord; SMP: superior medial protocerebrum; SP: subpallium; SRN: superior reticular nucleus; Tel: telecephalon; Vd: dorsal nucleus of ventral telencephalic area; VTA: ventral tegmental area; Vv: ventral nucleus of ventral telencephalic area.

response, but have not yet been investigated in any detail in this context.

In vertebrates, there appear to be conserved interactions between the telencephalon and mesencephalon. In mammals, encoding reward and directing motivated behaviors requires the coordination of multiple brain regions including the NAc, AMYG, PFC, substantia nigra (SN), ventral tegmental area (VTA), dorsal raphe nucleus (DRN), and the lateral habenula (LHb) (Figure 1(C)). The connections between these regions and others define the reward circuitry in the mammalian brain. Further, these connections appear to be conserved between rodents and primates (Figure 1(D)). In zebrafish, similar circuitry exists between the habenula (Hb), dopaminergic diencephalic cluster (DDC), interpeduncular nucleus (IPN), raphe nucleus (RN), and locus coeruleus (LC) (Figure 1(B)) (Ma, 1994; 2003; Rink & Guo, 2004; Rink & Wullimann, 2001; 2002; 2004; Tay, Ronneberger, Ryu, Nitschke, & Driever, 2011). It is important to note that although, the mesencephalic SN and VTA are the most prominent dopamine systems in mammals, these mesencephalic dopamine neurons are absent in zebrafish. The functional implication of this is unclear. Interestingly, recent work in the lamprey, one of the oldest vertebrate species, show that homologs of the mammalian HA and their efferent circuitry with dopamine and serotonin systems are remarkably conserved (Stephenson-Jones, Floros, Robertson, & Grillner, 2012). The extent to which this circuitry is functionally conserved, particularly in the context of reward, remains to be determined.

Use of multiple transmitters and brain regions imply that reward circuitry is undeniably complex in all brains. Recent advances in mammalian systems highlight that the neuronal populations within reward system brain regions are incredibly heterogeneous (Margolis, Toy, Himmels, Morales, & Fields, 2012; Nair-Roberts *et al.*, 2008). Furthermore, the full projections for most of these neurons are not characterized, leaving out an important dimension of complexity. In order to gain a complete understanding, one needs to have a clear identity of complete neuronal projections, genetic identity of these neurons, and of receptors expressed within a circuit. This is one of the ways in which invertebrate models may inform reward responses in more complex brains.

A centralized role of dopamine in encoding valence

Regardless of how simple the nervous system, an animal's survival depends on its ability to accurately encode memories of its experiences in order to successfully guide future behavior. These adaptive memories include the context of the experience, the associated cues, and the perceived valence, positive or negative. Dopamine neurons are capable of encoding both reward and aversion (positive and negative valence) and play a key role in reinforcing adaptive behavior. Their ability to attribute motivational salience to otherwise neutral stimuli is essential for the ability to remember stimuli and events in order to predict future behavioral solutions.

The role of dopamine in reinforcement learning is similar across phyla. In invertebrates, this has been most prominently studied in *Drosophila*, where dopamine plays a prominent role in both aversive and reward (appetitive) memory. Recent studies suggest that dopamine biases valence of cues

from the environment by integrating information about the unconditioned stimulus driving the behavioral response (Aso *et al.*, 2014b; Waddell, 2013). Activation of a subset of dopamine neurons (protocerebral anterior medial or PAM) can substitute for reward (Burke *et al.*, 2012; Liu *et al.*, 2012). Likewise, activation of subsets of dopamine neurons (posterior inferiorlateral protocerebrum or PPL1) can substitute for an aversive stimulus (Aso *et al.*, 2010; 2012; Claridge-Chang *et al.*, 2009; Schroll *et al.*; 2006). These two clusters of dopamine neurons fall into 20 dopamine neuron types that project axons to one, or at most two, compartments along axons of the mushroom body (Figure 2).

Dopamine released from these neurons is thought to bind to dopamine receptors in the axons of intrinsic mushroom body neurons (Kenyon cells) (Kim, Lee, & Han, 2007), which induces local changes in Kenyon cells (Boto, Louis, Jindachomthong, Jalink, & Tomchik, 2014; Cohn, Morantte, & Ruta, 2015). Dopamine-1-like and dopamine-2-like receptors are both required for learning and memory in *Drosophila* (Berry, Cervantes-Sandoval, Chakraborty, & Davis, 2015; Kim *et al.*, 2007; Qi & Lee, 2014; Shuai, Hu, Qin, Campbell, & Zhong, 2011). It is unclear, however, whether aversive or appetitive input results in differential dopamine receptor activation, as seen in mammals. Dopamine binding to receptors is thought to result in changes in activation along compartments of mushroom body axons, which affects mushroom body output neuronal (MBONs) response (Cohn *et al.*, 2015; Hige, Aso, Rubin, & Turner, 2015) (Figure 2). Convergence of dopamine neuron axons on compartmentalized Kenyon cell–MBON synapses creates a highly ordered unit that can support learning to impose valence on sensory representations (Figure 2).

In mammals, an intriguingly similar system is paralleled in the midbrain dopamine projections to the basal ganglia. In rodents, accruing evidence supports two separate dopamine directed pathways that have opposing behavioral results (Lobo & Nestler, 2011, Nakanishi, Hikida, & Yawata, 2014). The direct pathway activates dopamine-1 receptors located on medium spiny neurons, which increases their excitability and promotes behaviors that result in rewarding outcomes, whereas the indirect pathway activates dopamine-2 receptors on medium spiny neurons, which decreases their excitability and promotes behaviors that result in avoiding punishments. Receptor activation is reported to be dependent on the levels of extracellular dopamine; increases in dopamine levels activate dopamine-1 receptors and promotes reward learning,

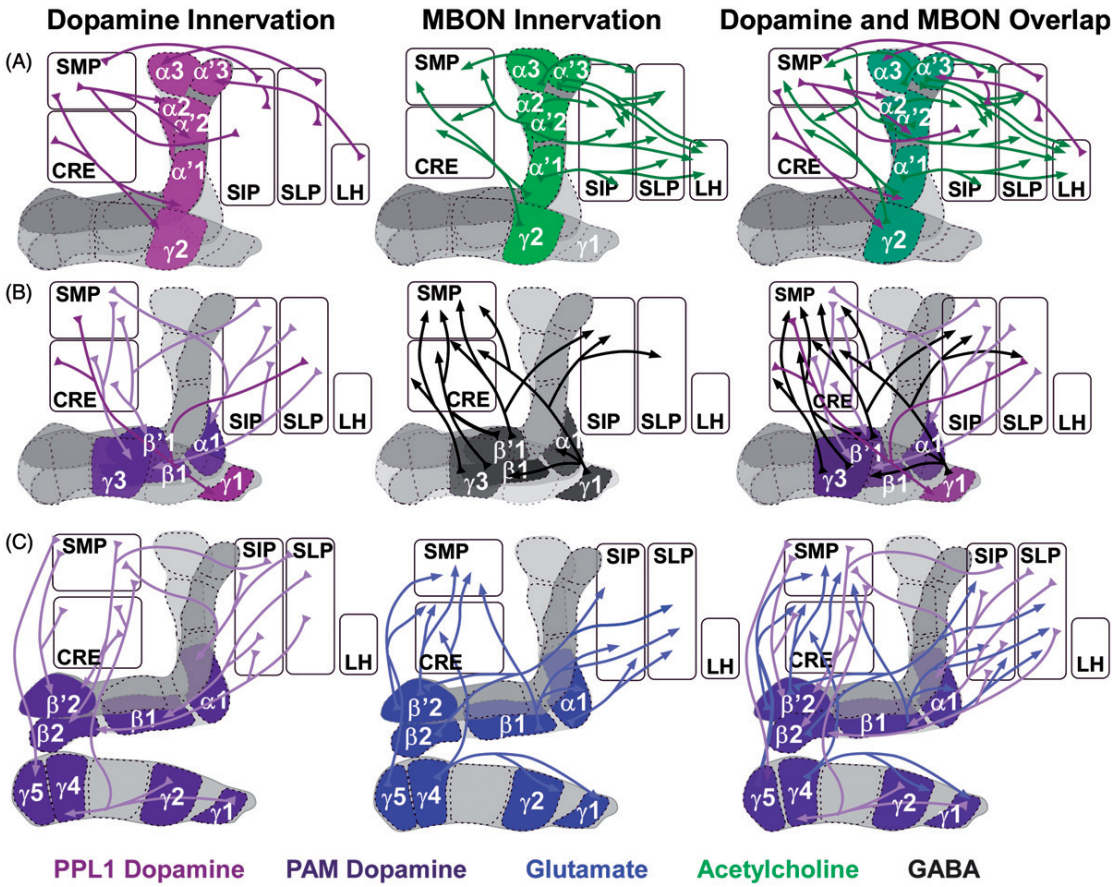

Figure 2. *Drosophila* mushroom body (MB) innervation patterns. The *Drosophila* MB comprise Kenyon cell axons that are segregated into three anatomically distinct lobes, the α/β, α/β, and γ outlined in gray) and is compartmentalized based on its innervation pattern. A. Dopamine–acetylcholine circuits. B. Dopamine–GABA circuits. C. Dopamine–glutamate circuits. Subsets of dopamine neurons (left) innervate the same MB compartments as output neurons (middle) and many of these neurons extend axons to the same anatomical regions where the dendrites of dopaminergic neurons are found creating opportunity for putative feedback circuits (right). Abbreviations: CRE: crepine neuropil; LH: lateral horn; PAM: dorsomedial anterior protocerebral dopamine cluster; PPL1: posterior inferiorlateral protocerebrum dopamine cluster; SIP: superior intermediate protocerebrum; SLP: superior lateral protocerebrum; SMP: superior medial protocerebrum.

whereas low levels of dopamine activate dopamine-2 receptors to promote avoidance learning (Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; Kravitz, Tye, & Kreitzer, 2012; Yawata, Yamaguchi, Danjo, Hikida, & Nakanishi, 2012). Evidence for this differential dopamine-dependent positive and negative reinforcement learning exists in both rodents as well as humans (Cox *et al.*, 2015). It is not yet clear whether this also exists in *Drosophila*. Intriguingly the number of neurons in the PAM cluster is significantly larger than the number of neurons in the PPL1 cluster, which might contribute to the extracellular dopamine levels and receptor activation. Supporting this observation is the finding demonstrating that small subsets of PAM neurons are required for shock memory (Aso *et al.*, 2010; 2012). Understanding how activation of different dopamine clusters in the *Drosophila* brain influences dopamine receptor activation will inform how functionally similar these neurons are.

Still lacking from this view, however, is the complex interactions of dopamine with other biogenic amines and neurotransmitters, the heterogeneity of neurons within these regions, and the complexity of receptor expression. Gamma-aminobutyric acid (GABA), opioid peptides, serotonin, acetylcholine, endocannabinoids, and glutamate are also implicated in acute reinforcing properties in mammals. Currently the standard account of how dopamine modulates reward learning involves dopaminergic modulation of cortical or limbic glutamatergic inputs onto GABAergic medium spiny neurons. More recent models have begun to incorporate the modulatory role of acetylcholine (Ashby & Crossley, 2011, Franklin & Frank, 2015; Tan & Bullock, 2008).

We propose that understanding how memories are formed in the well-characterized *Drosophila* mushroom body can be highly informative for understanding the central role of dopamine in reinforcement and in driving motivational response. In this system, dopamine release initiates a response within a complex network of neurons thought to work in a concerted fashion to assign valence to a stimulus (Aso *et al.*, 2014a; 2014b; Han, Millar, Grotewiel, M.S., & Davis, 1996; Kim, Lee, Seong, & Han, 2003) (Figure 2). These MBONs express glutamate, acetylcholine, or GABA, and together collectively bias behavior by conveying valence of the learned stimuli, irrespective of the modality of the stimulus or the specific reward or punishment used during learning (Aso *et al.*, 2014b).

Recent studies have identified this system as useful for understanding how internal state can shift an animal's evaluation of the appetitive nature of a stimulus, and resultantly, its behavioral choice to move toward or away from a stimulus. In *Drosophila*, the internal state of the animal, such as its state of food deprivation, appears to affect the physiology of select mushroom body compartments, thereby mediating the balance between positive and negative valence, and ultimately driving the output response (Lewis *et al.*, 2015). Here, we discuss similarities and differences between invertebrate and mammalian dopamine circuits required for assigning valence. We will attempt to distinguish between circuits that underlie motivation and those that encode reward, but also recognize that these circuits likely interact, perhaps in complex ways.

Dopamine–noradrenaline/octopamine circuits

Early studies in rodents identified noradrenaline (norepinephrine) as an important modulator of reward, particularly in the context of drug reward and feeding behaviors. For instance, operant paradigms using the self-administration of electrical impulses were most successful when electrodes targeted the noradrenergic system (Crow, 1972; Ritter & Stein, 1974). Margules, (1969) further reported that self-stimulation near the dorsal tegmentum, presumably the ascending noradrenergic fibers of passage, is enhanced by induced noradrenaline release and reduced by noradrenergic blockers. Early studies also showed that noradrenaline administration increased feeding in rats with lateral hypothalamic damage; an effect that was reversed by noradrenergic receptor blockers (Berger, Wise, & Stein, 1971). However, in recent years noradrenaline's role in reward has been overshadowed by the role of dopamine and thus has received considerably less attention.

Noradrenaline and the invertebrate neurotransmitter octopamine are both structurally and functionally similar (Roeder, 1999; 2005). Octopamine has a long-standing role in reward in invertebrates. Originally it was thought that dopamine and octopamine were part of separate motivational systems and had distinct roles in reward processing: octopamine was necessary for reward and dopamine was necessary for aversion (Aso *et al.*, 2010; Claridge-Chang *et al.*, 2009; Farooqui, Robinson, Vaessin, & Smith, 2003; Hammer & Menzel, 1998; Riemensperger, Voller, Stock, Buchner, & Fiala, 2005; Schwaerzel *et al.*, 2003; Tomchik and Davis 2009; Unoki *et al.*, 2005; Vergoz *et al.*, 2007). It is now clear that dopamine and octopamine/noradrenaline are both involved in processing rewarding and aversive stimuli and interact at many levels (Mizunami, Hamanaka, & Nishino, 2015; Perry & Barron, 2013; Waddell, 2013).

Maldonado and colleagues first challenged the idea that dopamine and octopamine are exclusively involved in aversive and appetitive processing, respectively. They demonstrated in crab *Chasmagnathus* that not only was octopamine necessary for appetitive memory formation, but it was also involved in aversive memory (Kaczer & Maldonado, 2009), which has subsequently been confirmed in honeybees (Agarwal *et al.*, 2011). Further investigation in crabs revealed that octopamine administration also impaired aversive memory reconsolidation (Kaczer & Maldonado, 2011) and dopamine administration impaired the formation of long-term appetitive memory (Klappenbach, Maldonado, Locatelli, & Kaczer, 2012).

More recently, compelling evidence in *Drosophila* outlines a role for octopamine and dopamine in appetitive and aversive memories as well as an interaction between both amines (Figure 3). Wu, Shih, Lee, & Chiang, (2013) showed that octopamine, released from the anterior paired lateral neuron (APL) innervating the mushroom body, was required for the consolidation of 3-hour aversive olfactory conditioning in *Drosophila* (Figure 3). Though this neuron is both GABAergic and octopaminergic, only reducing octopamine not GABA levels in the APL and only octopaminergic receptors in the mushroom body affected memory. Interestingly, a

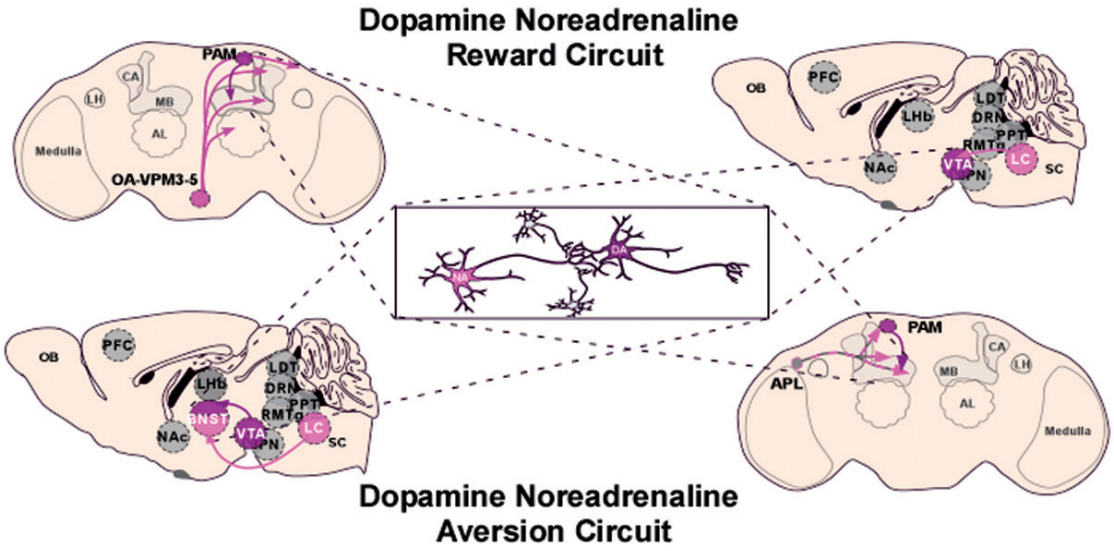

Figure 3. Dopamine–noradrenergic circuits implicated in reward and aversion. Top: dopamine–noradrenergic reward. In *Drosophila* octopamine-dependent sugar reward memory is mediated by OAMB located on dopaminergic neurons. This circuit includes the dopamine PAM cluster, the OA-VPM 3–5 neurons and requires the α-adrenergic-like OAMB receptor (Burke *et al.*, 2012). Similarly, in mammals, noradrenergic projections to dopamine neurons are essential for ethanol reward. In this LC-VTA circuit, noradrenergic LC projection neurons synapse on VTA dopamine neurons (Shelkar *et al.* 2015). It is thought that this circuit is bidirectional (not shown). Bottom: dopamine–noradrenergic Aversion. In *Drosophila*, a presumed dopamine–octopamine circuit includes the APL neuron, which co-expresses GABA and octopamine and innervations the MB underlies aversive memory. It is unclear whether APL directly synapses on PAM neurons, however, both of these neurons innervate the MB (Wu *et al.*, 2013). In mammals, noradrenergic projections have also been implicated in processing aversive stimuli. In the dorsal portion of the bed nucleus stria terminalis (BNST) aversive stimuli activate noradrenergic signaling, but inhibits dopaminergic signaling in the ventral BNST (the reverse is true for appetitive stimuli; Park *et al.* 2011). It is unclear whether noradrenergic and dopaminergic projections interact directly in the BNST. Gray neurons represent unidentified neurons that likely contribute to this reward micro-circuit. Abbreviations: DA: dopamine; NA: noradrenaline/octopamine.

previous study showed that reduced levels of GABA on the APL impaired learning (Liu & Davis, 2009), whereas in this study the effects of reducing octopamine was restricted to memory and not learning. Together these studies highlight the complex roles of individual neurons in different aspects of learning and memory.

Like noradrenaline, octopamine is also implicated in regulating feeding behaviors suggesting the conservation of function across species. In food-deprived *Drosophila* larvae, octopaminergic neurons, which express tyrosine decarboxylase, were targeted and manipulated. When these neurons were stimulated, larvae increased feeding behaviors, whereas when they were inhibited, feeding behaviors ceased (Zhang, Branch, & Shen, 2013). In *C. elegans* octopamine is released in the absence of food (Suo, Culotti, & Van Tol, 2009). When food is present, however, dopamine suppresses octopamine signaling through D2-like receptors, suggesting that these pathways are not separate.

Recently, Burke *et al.*, (2012) provided the most compelling evidence for an interaction between octopamine and dopamine in memory for the sweet taste of sugar (Figure 3). The authors used thermogenetics to show that formation of octopamine-dependent memory required the activation of specific dopaminergic neurons that innervate the mushroom body. The application of octopamine on exposed fly brains triggered an increase in intracellular calcium in these dopaminergic neurons as measured with GCaMP. Most striking, the direct thermogenetic activation of these octopaminergic neurons in the absence of the sugar reward was sufficient to form appetitive memories suggesting that these neurons are both necessary and sufficient for sugar memory. Further investigation revealed that octopamine from these neurons released onto a subset of dopamine neurons expressing the octopamine receptor (OAMB) convey the short-term reinforcing effects of sweet taste (Huetteroth *et al.*, 2015).

In insects and mammals, noradrenergic/octopaminergic projections are widely distributing through the brain, suggesting a prominent modulatory role. In mammals noradrenergic projections target regions include the hippocampus, AMYG, and the VTA. However, its role in reward processing has been historically underscored. Recently, a few studies have brought noradrenaline to the forefront. For example, using a pharmacological approach, Velasquez-Martinez, Vazquez-Torres, & Jimenez-Rivera, (2012) demonstrated that activation of alpha1-adrenoreceptors enhances glutamate release onto VTA dopamine cells. Pharmacological approaches have also shown that noradrenaline is an important regulator of the LHb via dopamine D4 receptors (Root *et al.*, 2015). Given this enticing evidence and the predominant role that octopamine plays in invertebrates, the role of noradrenaline modulating dopaminergic reward processing, should be reconsidered.

Dopamine–glutamate circuits

Glutamate is an integral part of all reward circuits, but our understanding of how it interacts with dopaminergic circuits in the context of reinforcement learning is in its infancy. In *Drosophila*, Ichinose *et al.*, (2015) recently identified a recurrent reward circuit that includes a single class of PAM dopaminergic neurons and the glutamatergic output neurons innervating the α1 compartment of the mushroom body. The

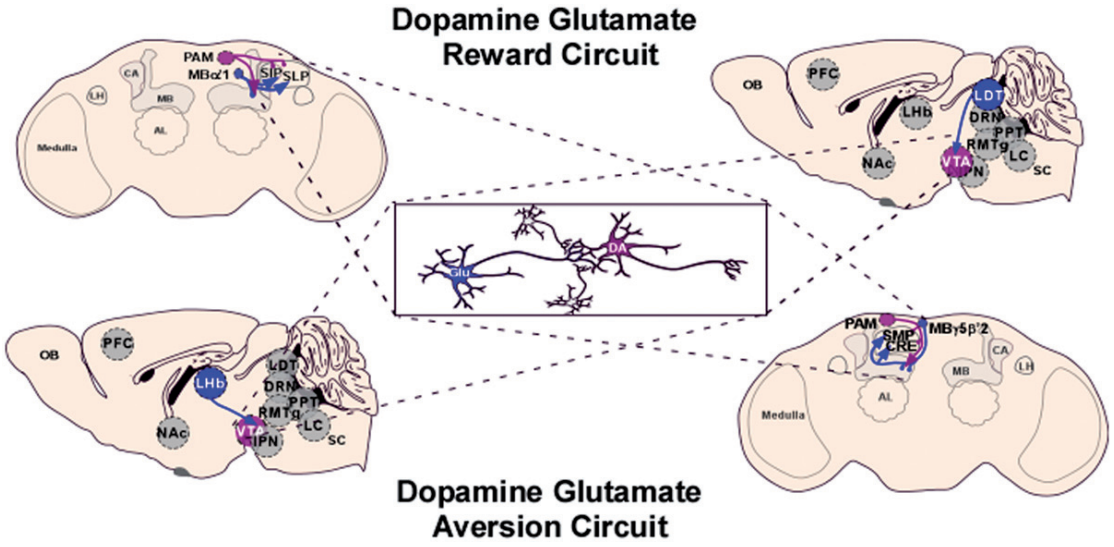

Figure 4. Dopamine–glutamate circuits implicated in reward and aversion. Top: dopamine–glutamate reward. In *Drosophila*, interactions between a subset of dopamine PAM cluster neurons and the MB α1 glutamate output neurons underlie sugar reward memory (Ichinose *et al.*, 2015). These glutamatergic neurons extend axons to the superior intermediate protocerebrum (SIP) and superior lateral protocerebrum (SLP) regions where dendrites of the PAM neurons are found. A similar reward circuit was identified in rodents, which include glutamatergic input from the LDT to the VTA (Lammel, Ion, Roeper & Malenka, 2012). These targeted VTA dopaminergic neurons project to the lateral shell of the NAc (not shown). Bottom: dopamine–glutamate aversion. In *Drosophila*, activation of the M4-6 neurons, which include the MBγ5β′2 glutamatergic output neurons induced odor-driven avoidance (Owald *et al.*, 2015). Interestingly, in a separate study activation of the PAM dopaminergic neurons innervating this MB compartment reduced innate CO_2 avoidance (Lewis *et al.*, 2015). A similar aversion circuit was identified in rodents, which include glutamatergic input from the lateral habenula to the VTA (Lammel *et al.*, 2012). These subsets of VTA neurons project to the prefrontal cortex and RmTg (not shown). Activation of these neurons induced conditioned place avoidance. These circuits are presumed to also be present in primates, however, have yet to be confirmed as indicated by the dotted line. Gray neurons represent unidentified neurons that likely contribute to this aversion micro-circuit. Abbreviations: DA: dopamine; Glu: glutamate.

interactions between these neurons and the α/β mushroom body Kenyon cells (via DopR1 receptors) are critical for 24-hour sugar reward memories. Using GFP reconstitution across synaptic partners (GRASP), the authors show that the dendrites of the dopaminergic neurons and the presynaptic terminals of the glutamatergic neurons lie in close apposition within the SIP, SLP, and mushroom body (Figure 4). They also report the presence of NMDAR in the dendrites of the dopaminergic neurons in SIP and SLP (Ichinose *et al.*, 2015).

Importantly, this study provides *in vivo* behavioral analysis that highlights how a feedback loop between dopamine, mushroom body neurons and a glutamate neuron drive acquisition and consolidation of appetitive memory. Glutamate neurons modulate dopaminergic neurons activity via NMDA receptors and dopaminergic neurons modulate Kenyon cell activity via D1 receptors. This recurrent activity likely provides a mechanism for the development of long-term memory. In particular, ongoing activity selectively enhances the gain of a reward signal such that when relevant cues are reintroduced, memory is retrieved.

This feedback loop may be informative for understanding how VTA dopamine neurons regulate conditioned place preference in mammals (Figure 4). Interestingly, Lammel *et al.*, (2012) report conditioned place preference behavior following activation of VTA glutamatergic input from the laterodorsal tegmentum (LDTg). These VTA dopaminergic neurons preferentially synapse on neurons in the lateral shell of the NAc. The LDTg is a known target of VTA dopaminergic neurons (Cornwall, Cooper, & Phillipson, 1990), however, it appears that this may not be a monosynaptic feedback loop (Lammel *et al.*, 2012) (Figure 4). Similarly, activation of glutamate neurons from the DRN to the VTA is sufficient to reinforce behavior in mice (McDevitt *et al.*, 2014). Early studies describe VTA dopaminergic projections to the DRN, however these have yet to be functionally explored (Mendlin, Martin, & Jacobs, 1999; Peyron *et al.*, 1995).

The activation of glutamatergic neurons does not always signal reward (Figure 4). Owald *et al.*, (2015) described the requirement of a subset of glutamatergic neurons (M4-6) for the expression of appetitive and aversive memory in *Drosophila*. Similar to VTA glutamatergic input from the LHb (Lammel *et al.*, 2012), activating these neurons induced avoidance behavior, whereas silencing the output of these neurons was sufficient to change a previously associated odor-driven avoidance into an approach (Aso *et al.*, 2014b; Owald *et al.*, 2015) (Figure 4). What is particularly compelling about the work of Owald *et al.*, (2015) is that the inactivation of M4-6 glutamatergic neurons impairs the expression of both a learned appetitive and aversive memory. This suggests that the output of these neurons is modulated by experience. Given that in rodent models, artificial activation of neurons are almost exclusively performed within behaviorally naïve animals, it would be of interest to see how experience modulates the involvement of these neurons. In flies, the anatomy and odor tuning of M4-6 glutamate neurons suggests that these neurons pool odor-driven Kenyon cell synaptic outputs and bidirectionally regulate memory (Owald *et al.*, 2015). Similar synaptic pooling could take place in mammalian circuits.

Whether these glutamate neurons feed back onto PAM dopamine neurons, like the α1 loop above remains to be resolved. Aso *et al.*, (2014a) showed that these M4-6 glutamatergic output neurons extend their axons to two separate

locations where the dendrites of dopaminergic neurons innervating the mushroom body can be found, suggesting a putative feedback circuit between PAM dopaminergic neurons and M4-6 glutamatergic neurons. However, it is still unclear if these are actual monosynaptic feedback loops or two separate parallel pathways [recent GRASP failed to show a connection (Lewis *et al.*, 2015)]. Future techniques may elucidate the existence of this potentially powerful feedback circuit for investigation of the modulation of directed behavior.

We posit that a similar mechanism may occur between LHb and VTA connections in mammals. Lammel *et al.*, (2012) showed that glutamatergic LHb neurons synapse on VTA dopaminergic neurons, that preferentially project to the medial PFC as well as GABAergic neurons in the RMTg. Optogenetic activation of these neurons elicited strong conditioned place aversive behaviors. (Lammel *et al.*, 2012) (Figure 4). Interestingly, these are not asymmetrical inputs: the LHb projects to the VTA, both directly and indirectly through GABAergic neurons in the RMTg, and the VTA projects back to the LHb to modulate activity (Lammel *et al.*, 2012; Root, Mejias-Aponte, Qi, & Morales, 2014).

Clearly, there are many dopamine–glutamate circuits that regulate both positive and negative reinforcement, solely due to the abundance of glutamate and its function as a fast-acting transmitter. However, two seemingly similar dopamine to glutamate connectivity patterns appear to act in similar ways to regulate appetitive and aversive responses across species. This suggests that the well-defined circuits in the fly could inform the more complex mammalian circuits.

Dopamine–acetylcholine circuits

Acetylcholine is one of the first described neurotransmitters (Loewi, 1921). Given the pervasiveness of cholinergic signaling in the brain, it is not surprising that it plays an integral role in motivated behaviors. However, its precise role in modulating learning and memory and reward in the brain is less developed (Aosaki *et al.*, 1994; Inglis, Olmstead, & Robbins, 2001; Joshua, Adler, Mitelman, Vaadia, & Bergman, 2008; Morris, Arkadir, Nevet, Vaadia, & Bergman, 1994; Morris, Arkadir, Nevet, Vaadia, & Bergman, 2004; Okada, Toyama, Inoue, Isa, & Kobayashi, 2009; Okada & Kobayashi, 2013).

In the mammalian brain, there are several loci that provide cholinergic input including the LTDg, the pedunculopontine nuclei (PPTg), and the basal forebrain. Cholinergic input to the VTA primarily originates in the LTDg (Cornwall *et al.*, 1990), whereas input to the SN primarily originates in the PPT (Oakman, Faris, Kerr, Cozzari, & Hartman, 1995). The LTDg input plays a major role in regulating the activity of dopamine neurons (Gronier & Rasmussen, 1998; Kitai, Shepard, Callaway, & Scroggs, 1999; Maskos, 2008) and has recently been linked to drug-associated memories (Dobbs & Cunningham, 2014; Solecki *et al.*, 2013, Shinohara, Kihara, Ide, Minami, & Kaneda, 2014; Witten *et al.*, 2010). However, the exact mechanisms through which these circuits function to induce changes in appetitive behavior are unclear.

We hypothesize that specific dopamine–acetylcholine circuits required for memory in *Drosophila* can inform these complicated LTDg acetycholine to VTA dopamine circuits. In flies, activation of ensembles of cholinergic MB output neurons induces preference, and is required for multiple forms of appetitive memory, and long-term aversive memory (Aso *et al.*, 2014b). Learning either an appetitive (Placais, Trannoy, Friedrich, Tanimoto, & Preat, 2013) or aversive (Pai *et al.* 2013) association increases activity of cholinergic MB α3 output neurons (Placais *et al.*, 2013). These neurons appeared to be specifically involved in expression memories. Intriguingly, the MB α3 output neuron is innervated by a dopamine neuron from the PPL1 cluster, and appears to project back to dendrites of this same neuron creating a putative feedback loop (Aso *et al.*, 2014b). This feedback loop suggested a gain control for regulating expression, and perhaps consolidation, of memory.

Likewise, the MB α2 output neuron is also required for aversive memory expression. However, learning an aversive association decreases, rather than increases, the response in the cholinergic MB α2 output neuron (Sejourne *et al.*, 2011). Similar to the MB α3 output neuron, the MB α2 receives input from a PPL1 dopamine neuron (Aso *et al.* 2014b). However, unlike the MB α3 output neuron, the MB α2 output neuron appears to project onto dendrites of PPL1 dopamine neurons that innervate the α1 and α'3 compartments. This suggests a feed-forward network, which may aid in ensemble actions of cholinergic MB output neurons in driving a behavioral response.

Thus, in flies it appears as if major cholinergic MB output neurons receive input from dopamine neurons, and may feedback onto those same dopamine neurons, or project in a feed-forward manner to other dopamine neurons that, in turn, project onto neighboring cholinergic MB output neurons. Whether the LDTg to VTA circuit shows a similar connectivity is currently unknown. Genetic tools providing increased spatial resolution will help resolve the connectivity.

Dopamine–GABAergic circuits

GABAergic neurons constitute the main type of inhibitory neuron in the adult mammalian brain and play a critical role in regulating neuronal excitability. The majority of these neurons have been identified as local inhibitory neurons, however, more recent work has also described long-range projection GABAergic neurons (Caputi, Melzer, Michael, & Monyer, 2013). There are several notable regions where GABAergic neurons reside that are relevant to this review: the lateral hypothalamus, the VTA, the NAc, and the RMTg. In the VTA, GABAergic neurons are the second largest population comprising approximately 30% and have an identified role in modulating local dopaminergic activity, as well as the activity in other brain regions.

There is mounting evidence that GABAergic neuronal function is evolutionarily conserved from flies to vertebrates. For instance, recent *Drosophila* work has identified a role for GABAergic neurons in promoting sleep, similar to some mammalian GABAergic populations (Brown & McKenna, 2015; Haynes, Christmann, & Griffith, 2015). *Drosophila* GABAergic neurons have also been reported to negatively regulate neuronal activity. Lin *et al.*, (2014) showed that

Kenyon cells activate the GABAergic APL and this neuron subsequently inhibits Kenyon cell activity. Interrupting this feedback loop disrupts discrimination of similar odors. Further, ingestion of the GABA agonist Gaboxadol, leads to the reduction of dopaminergic activity, which is critical for memory retention (Berry *et al.*, 2015).

Flies also show preference for optogenetic stimulation of different GABAergic MBONs suggesting a critical role for GABA in appetitive responses. These neurons project directly to populations of dopamine neurons that when activated, substitute for reward (Aso *et al.*, 2014b). Like most other MBONs, however, these highly interconnected neurons likely act in an ensemble fashion to shift the valence of an output response. In support of this, inactivation of other GABAergic neurons that innervate the γ peduncle of the mushroom body reduced aversive shock memories. These GABAergic neurons project to dopamine PAM neurons, which appear to project to the γ3 and β1 MB compartments, however they are innervated by a dopamine PPL1 γ neuron. Intriguingly, a neighboring GABAergic MB output neuron has its dendrites in the γ3 MB compartment. The anatomical organization of these neurons implies a dopamine-GABA feed-forward network that may result in GABA "reward" neurons negatively regulating selective populations of dopamine neurons.

Similarly, recent work in the mammalian field supports the hypothesis that VTA GABAergic neurons regulate reward learning by negatively regulating some populations of dopaminergic neurons that encode reward. Previous work identified two subpopulations of VTA neurons that have opposing responses to aversive stimuli. Non-dopaminergic neurons tended to increase their activity, whereas dopaminergic neurons reduced their activity (Ungless, Magill, & Bolam, 2004 but Brischoux, Chakraborty, Brierley, & Ungless, 2009; Lammel, Ion, Roeper, & Malenka, 2011; Lammel *et al.*, 2012). More recently, Tan *et al.*, (2012) identified some of these non-dopaminergic neurons as GABAergic and showed that their activation suppresses dopaminergic activity in the VTA. Further, they report that the optogenetic activation of VTA GABAergic neurons is sufficient to drive avoidance behaviors. Using a different Cre driver line (VGAT-Cre), van Zessen, PHillips, Budygin, & Stuber, (2012) also targeted GABAergic neurons in the VTA and showed that *in vivo* optogenetic activation results in strong inhibition of neighboring dopaminergic neurons. Interestingly, they show that this activation results in the cessation of appetitive response behaviors.

These two mammalian studies are particularly compelling because they demonstrate a local dopamine-GABA circuit within the VTA, where GABAergic neurons directly regulate the excitability of subpopulations of dopaminergic neurons and their activity underlies aversion behaviors. Conversely, inhibiting VTA GABA neurons disinhibits VTA dopamine neurons, inducing an appetitive response (Jennings *et al.*, 2013). Together, these experiments imply a functional connectivity not unlike the *Drosophila* GABA–dopamine connectivity. Perhaps GABA–dopamine mammalian circuits also work in a feed-forward manner as the *Drosophila* circuits imply.

Dopamine–serotonin circuits

Serotonin is evolutionarily one of the oldest neuromodulators (Peroutka & Howell, 1994). It has widespread projections in insect and mammalian brains, conserved biosynthetic pathways, and are thought to modulate reward among other behaviors. The mammalian DRN is the largest serotonergic nucleus in the brain; consistent with its role in reward, its efferents target the VTA and NAc where serotonergic receptors are localized (Bubar, Stutz, & Cunningham, 2011; Herve, Pickel, Joh, & Beaudet, 1987; Nocjar, Roth, & Pehek, 2002). Early studies showed that local infusions of serotonergic receptor agonists stimulated DA release in the rodent VTA (Campbell, Kohl, & McBride, 1996; Liu, Thielen, Rodd, &

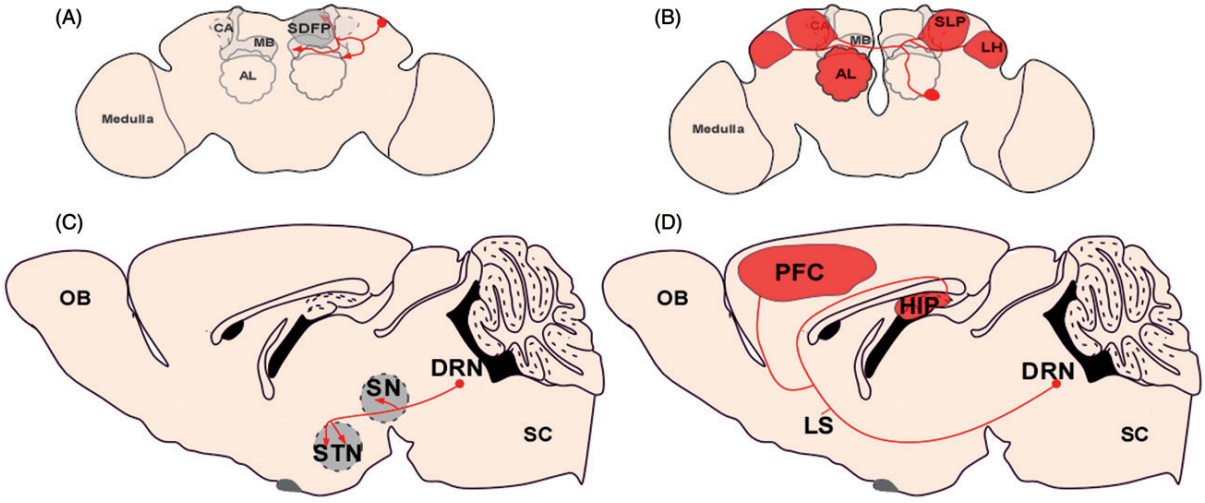

Figure 5. Similar neuron complexity across species. (A) Example serotonin neuron in fly brain with discrete projections to the mushroom body and superior dorsofrontal protocerebrum (Chiang *et al.*, 2011). (B) Example contralaterally projecting serotonin-immunoreactive deutocerebral (CSD) neuron. This neuron has broad and extensive innervation pattern in the fly brain. Its dendrites (not shown) innervate the antennal lobe in one hemisphere and its axons project to the lateral horn (LH), superior lateral protocerebrum (SLP), and the contralateral antennal lobe. The CSD neuron has extensive arborizations in these regions as depicted by the areas shaded red (Chiang *et al.*, 2011). (C) Example serotonin neuron recently reconstructed in the mouse brain with discrete projections to the substantia nigra (SN) and the subthalamic nuclei (STN) (Gagnon & Parent, 2014). (D) Example serotonin neuron recently reconstructed in the mouse brain with extensive arborizations in the prefrontal cortex and the hippocampus as depicted by the areas shaded in red (Gagnon & Parent, 2014).

McBride, 2006); thus, an obvious circuit motif within reward/motivation systems is serotonin and dopamine.

Serotonin plays an important, but highly complex role in modulating motivated behaviors. The sheer diversity of serotonergic neuronal populations and receptors makes studying this role with high spatial, temporal or functional precision particularly difficult. Invertebrate models could thus be especially helpful in identifying and functionally separating these discrete populations. As with all models, some features may not be conserved although serotonergic circuits appear to carry similarities in complexity (Figure 5). For example, co-expression of serotonin and GABA has been described in the RN in rodents and lamprey and in the DPM neuron in flies (Barreiro-Iglesias, Cornide-Petronio, Anadon, & Rodicio, 2009; Fu *et al.*, 2010; Lee *et al.*, 2011). Identifying the various circuit motifs and their functional implications is critical to understanding the complex and context-dependent role serotonin plays in modulating reward and motivation.

There appears to be a cross-species similarity in the ability of serotonin to mediate internal state, such as hunger. Serotonin may thus affect appetitive behavior by increasing the motivational response to receive reward: for example, by inducing hunger or blocking satiety. In *C. elegans*, the release of serotonin increases feeding behaviors (Avery & Horvitz, 1990; Croll, 1975) whereas the inability to synthesize serotonin results in a decrease in feeding behavior (Sze, Victor, Loer, Shi, & Ruvkun, 2000). The release of serotonin is thought to underlie increased feeding in response to familiar foods via the SER-7 receptor (Song, Faumont, Lockery, & Avery, 2013). Similar increases in feeding behaviors were reported in *Drosophila* when a subset of serotonergic neurons was thermogenetically activated (Albin *et al.*, 2015). Degrading tryptophan hydroxylase, a rate-limiting enzyme in the production of serotonin, in these neurons using RNAi demonstrated that serotonin release was responsible for the increase in feeding behavior, and suggested it was responsible for evoking feelings of hunger (Albin *et al.*, 2015). Intriguingly, this subset of serotonin neurons appears to project in the general vicinity of PAM dopamine neurons shown to be required for appetitive memory, although these projections have not been confirmed using genetic tools.

A role for serotonin in feeding behaviors also exists in vertebrates; however, there is disagreement as to whether serotonin is involved in signaling hunger or satiety (for a comprehensive review, see Voigt & Fink, 2015). In mice, subsets of DRN serotonergic neurons are phasically excited by either punishments or reward predicting cues (Cohen, Amoroso, & Uchida, 2015). However, whether these responses are associated with a consequent shift in internal state, or are directly associated with formation of the memory itself remains to be seen. In *Drosophila*, serotonin modulates both aversive and appetitive olfactory memory, which suggests it plays multiple roles in facilitating reinforcement (Johnson, Becnel, & Nichols, 2011; Lee *et al.*, 2011; Sitaraman, LaFerriere, Birman, & Zars, 2012). Together these data highlight that serotonin has a complex role in modulating behaviors in response to rewarding or aversive stimuli and its complexity is shared across species. It seems likely that the complex role of serotonin in motivated behavior is due to the heterogeneity of serotonin receptors in different structures and, perhaps, its interactions with dopamine.

Dopamine and neuromodulator peptides

Hormones and neuropeptides, like grehlin, leptin, insulin, and neuropeptide Y (NPY) have complex modulatory effects on motivational behaviors and reward circuitry. This is largely a result of their broad and complex innervation patterns, different time scales for neuronal activation and signaling, and diverse receptor types. Some neuropeptides and hormones have established modulatory roles in mammalian dopaminergic circuits (Cone, McCutcheon, & Roitman, 2014; Labouebe *et al.*, 2013; Opland, Leinninger, & Myers, 2010; Perry *et al.*, 2010).

It is likely that hormone and peptide regulation of dopamine signaling is functionally conserved. For example, NPY in mammals and the invertebrate analog neuropeptide F (NPF) regulate diverse motivational responses, and its actions are context-dependent. NPY acts as an orexigenic, increases motivation to receive reward, encodes reward, and decreases anxiety (Gilpin, 2012; Quarta & Smolders, 2014). In *Drosophila*, the form of NPF most similar to mammalian NPY (long NPF), regulates feeding, promotes memory performance in sated flies, encodes reward, and shifts the valence of appetitive stimuli (Krashes *et al.*, 2009; Rohwedder, Selcho, Chassot, & Thum, 2015; Shohat-Ophir, Kaun, Azanchi, Mohammed, & Heberlein, 2012; Wu *et al.*, 2003). Intriguingly, the role of NPY in appetitive behaviors also extends to zebrafish where it affects feeding and *C. elegans* where it affects the aggregation of worms on food (de Bono, Tobin, Davis, Avery, & Bargmann, 2002; Yokobori *et al.*, 2012).

Combined evidence between flies and rodents suggests that not only is the role of NPY shared across species, but so are the context-dependent circuit motifs through which it acts. In both flies and rodents, NPY may regulate behavior through dopamine circuits. In *Drosophila* larvae, NPF co-localizes with dopamine in neurons required for odor-induced feeding (Wang, Pu, & Shen, 2013). In *Drosophila* adults, downregulating the NPF receptor in dopamine neurons decreases the appetitive response to a cue previously associated with sucrose (Krashes *et al.*, 2009). In rodents, slice electrophysiology and microdialysis experiments have shown that NPY inhibits dopamine and GABA cells in the VTA and increases extracellular dopamine in the NAc (Ault, Radeff, & Werling, 1998; Korotkova, Brown, ergeeva, Ponomarenko, & Haas, 2006; Quarta, Leslie, Carletti, Valerio, & Caberlotto, 2011; Sorensen *et al.*, 2009). Interestingly, this NPY-dopamine circuit may also modulate noradrenergic signaling further diversifying its modulatory effects (Quarta *et al.*, 2011; Vahatalo, Ruohonen, Ailanen, & Savontaus, 2015). NPY also co-localizes with D1 receptor expressing cells in the centromedial AMYG (Wood *et al.*, 2015). Combined, this evidence implies a complex relationship between NPY and dopamine where, in addition to NPY modulating dopamine signaling, dopamine modulates NPY signaling.

Other neuropeptides such as FMRFamides, tachychinin, allatostatin, pigment-dispersing factor, diuretic hormone in

insects, and numerous neuropeptide-like proteins (NLP) in worms have diverse effects on behavior although direct homologs, orthologs, or paralogs have not yet been characterized. One reason for this is because the cross-species comparisons of these peptides can be difficult to establish by sequence homology. It is likely that further investigation may reveal direct similarities: for example, diuretic hormone may act as a stress hormone similar to corticotropin-releasing hormone, and worm NLP-24 has opioid-like effects on feeding (Cheong *et al.*, 2015, Cannell *et al.*, 2016). Understanding how these various neuropeptides and hormones regulate circuit motifs required for reward responses across species will be key to understanding how appetitive responses are mediated and how internal state and environment influences these responses.

Caveats in comparing circuits across species

Despite remarkable similarity between species, it is critical not to assume that more complex mammalian circuits function in an identical manner to invertebrate circuits and vice-versa. We still have very little understanding of the complete organization and architecture of fly and mouse brains, never mind a thorough understanding of how small circuit motifs work in concert. Further, the structure of invertebrate neurons is very different from that of mammalian neurons. For instance, *Drosophila* central brain neurons are unipolar; as such the cell body is physically separated from its processes. In mammals, some sensory neurons are unipolar neurons, however, the majority of neurons are classified as bipolar or multipolar. In both cases the cell body is situated between the dendrites and axon. This structural difference could have important implications for the physiology of the cell that is not immediately clear. Despite this structural difference, however, microtubule dynamics work in remarkably similar ways in both flies and mice (Rolls & Jegla, 2015).

Intriguingly, recent individual neuron reconstructions in rodents have revealed considerable similarity in dendritic and axonal arborization complexity to that of flies (Aransay, Rodriguez-Lopez, Garcia-Amado, Clasca, & Prensa, 2015; Gagnon & Parent, 2014; Schwarz *et al.*, 2015). For instance, some serotonin neurons in flies and mice appear to arborize in discrete regions, whereas others innervate more broadly (Figure 5). Similar innervation patterns position these neurons to have similar neuromodulatory roles across species. Likewise, dendritic and axonal arborization patterns of *Drosophila* and rodent dopamine neurons have a very similar organization (Aso *et al.*, 2014a; Matsuda *et al.*, 2009). The innervation patterns of midbrain dopamine neurons appear to compartmentalize the striatum in a manner reminiscent of how dopamine neurons compartmentalize the mushroom body in *Drosophila*. This suggests that dopamine circuits are organized in a very similar way in insects and mammals, and the differences in order in magnitude (~250 neurons compared to 25,000 neurons) may be simply more compartments, or larger subpopulations of neurons innervating a limited number of compartments.

Nonetheless, it is critical to avoid oversimplification in discussions of how circuits function in behavior. Clearly, invertebrates have substantially less neurons than rodents, thus the activity of suites of neurons in mice and single neurons in the *Drosophila* brain may not be functionally equivalent. Additionally, in all species, the diversity of receptors expressed post- and pre-synaptically are critical in determining circuit function. Indeed, analyzing circuit function in the absence of knowledge of the receptors expressed in the circuit is futile; whether the binding of a modulator results in an excitatory or inhibitory responses depends on the receptor to which it binds. Both flies and mice have a variety of receptors for each neuromodulator, and the structures of these receptors appear to be fairly conserved across species (Brody & Cravchik, 2000; Hen, 1992; Xia & Chiang, 2009). However, the function of these receptors in individual neurons in behaving animals in any organism is not yet well understood. New genetic tools in invertebrates like *C. elegans* and *Drosophila* only now make answering these questions feasible.

There is an added layer of complexity at the single cell level: neurons are highly complex and dynamic cells and co-express multiple neurotransmitters and peptides. How co-expression differs between species, however, is entirely unknown. Co-expression is particularly important because in the mouse VTA, the majority of individual neurons often co-express dopamine and GABA or dopamine and glutamate, further complicating circuit dynamics (for comprehensive reviews, see Pupe & Wallen-Mackenzie, 2015; Roeper, 2013). The co-expression of serotonin and GABA has been described across species (Barreiro-Iglesias *et al.*, 2009; Fu *et al.*, 2010), so it seems reasonable to suggest that this feature is conserved.

An additional complicating factor is that neurons can change the neurotransmitters they release (Spitzer, 2015). Thus, across all species, it is critical not to assume that functional circuitry characterized in one context will be the same for all contexts. Because neurons within circumscribed brain regions are so dynamic and heterogeneous, even within the same subtype, it is especially important to understand the neural circuitry with microcircuit resolution.

A comparative approach to understanding reward circuit function

Understanding how circuits encode reward and direct motivated behaviors has been an elusive pursuit of neuroscientists for decades. Recently it has become clear that much of this difficulty lies in: (1) developing an appreciation of the heterogeneity of interactions between neurons and (2) investigating *in vivo* responses of genetically identified neurons in behaving animals.

In order to comprehensively understand how reward circuitry drives motivational responses, it is critical to understand the activity patterns of different types and subtypes of neurons while animals perform various reward associated tasks and how these neurons interact. Of course, not all stimuli are inherently rewarding or averse; such is the case with many drugs of abuse. These experiences, and others, can be both rewarding and aversive and therefore require interplay between positive and negative reinforcement circuits. The complex

interaction between these circuits results in a context-dependent valence assignment and ultimately drives behavior.

Understanding the complexity of these circuitry interactions requires precisely defined circuits with great spatial resolution. As this type of resolution is currently limited in mammalian brains, invertebrate circuits may be very useful for informing our understanding of how circuit motifs function during rewarded behavior and decision-making that drives motivational response as well as how circuit motifs are modified to result in aberrant behavior. Thus, we argue that in order to develop a comprehensive understanding, scientists should look across animal species and capitalize on the unique perspectives they provide. This approach will inform the neural and molecular mechanisms underlying complex behavior disorders associated with motivational response such as depression, anxiety, and addiction.

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