Neuromodulatory pathways in learning and memory: lessons from invertebrates

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Abstract

In an ever-changing environment, animals have to continuously adapt behavior. The ability to learn from experience is crucial for animals to increase their chances of survival. It is therefore not surprising that learning and memory evolved early in evolution and are mediated by conserved molecular mechanisms. A broad range of neuromodulators, in particular monoamines and neuropeptides, have been found to influence learning and memory, but our knowledge on their modulatory functions in learning circuits remains fragmentary. Many neuromodulatory systems are evolutionarily ancient and well-conserved between vertebrates and invertebrates. Here, we highlight general principles and mechanistic insights on the actions of monoamines and neuropeptides in learning circuits that have emerged from invertebrate studies. Diverse neuromodulators have been shown to influence learning and memory in invertebrates, which can have divergent or convergent actions at different spatiotemporal scales. In addition, neuromodulators can regulate learning dependent on internal and external states, such as food and social context. The strong conservation of neuromodulatory systems, the extensive toolkit and compact learning circuits in invertebrate models makes these powerful systems to further deepen our understanding of neuromodulatory pathways involved in learning and memory.

1. Introduction

Learning is fundamental to the survival of animals, as it allows adaptation of behavior to environmental changes. Besides learning from experience, animals have also evolved the ability to store and retain this knowledge as memory. Given the adaptive advantage, it is not surprising that learning and memory evolved early in evolution and are mediated by common molecular mechanisms in vertebrate and invertebrate animals (1).

A prominent cellular process underlying learning and memory is synaptic plasticity, referring to changes in the effectiveness of synaptic transmission between neurons (i.e. synaptic strength). This plasticity is mediated by the action of neuromodulators, small signaling molecules that can modify neuronal properties, such as excitability and synaptic strength (2). In the last decades, many neuromodulators have been implicated in learning and memory, but we are only beginning to understand their functions in memory circuits (3,4). The two main groups of neuromodulators involved in learning are neuropeptides and monoamines, which typically act by binding to G protein-coupled receptors (GPCRs). Many of these signaling systems are ancient and highly conserved across vertebrates and invertebrates (e.g. 5). For several monoamines and neuropeptides, structural conservation appears to correlate with functional similarity in learning and memory (4,6,7). Over the past half-century, the rapid advance of genetic tools to study neuromodulation and the amenability of invertebrate learning circuits to implement these tools have deepened our understanding of neuromodulator actions in learning and memory. Here, we briefly review the classification and conserved molecular basis of learning and memory, before elaborating on neuromodulatory pathways in invertebrate learning circuits and correlating findings with studies in vertebrate models.

2. Evolutionary conservation of learning and memory

2.1. Classification of learning and memory

Learning and memory can be categorized based on different behavioral and molecular criteria that were initially introduced for vertebrate learning. At the behavioral level, memory is traditionally classified as explicit (declarative) or implicit (non-declarative) (**Figure 1**) (8). Explicit memory constitutes conscious learning, such as remembering facts (semantic memory) and events (episodic memory), and is almost exclusively studied in mammals (8). By contrast, implicit memory comprises learned skills, reflexes, habits and behaviors and has been described in many animals, including humans, mice, insects, mollusks and nematodes (1,2,9).

Procedural memory, i.e. the formation of motor memories, non-associative and associative learning are the main types of implicit memories (**Figure 1**) (10). Non-associative learning

covers behavioral changes after exposure to a particular stimulus. Animals can learn to respond less after repeated or prolonged exposure to a cue when it has no adverse effects (habituation). In contrast, exposure to a noxious or discomforting stimulus can increase behavioral responses to other stimuli, including non-noxious cues (sensitization) (2,10). Well-known examples of non-associative learning include habituation of the proboscis extension response in dipterans, tap habituation in *Caenorhabditis elegans*, habituation and sensitization of the gill-withdrawal reflex in *Aplysia*, and changes in mouse exploratory behavior in response to a new environment (11–14). In associative learning, on the other hand, a positive or negative association is formed between a conditioned and an unconditioned stimulus. Several conditioning procedures, such as classical and operant conditioning, have been described in vertebrate research (15). Classical (Pavlovian) conditioning occurs when animals change their response to a conditioned stimulus after it is paired with an unconditioned stimulus. In operant conditioning, also referred to as instrumental learning, the strength of a behavior is modified by reinforcement or punishment. Similar forms of associative learning have been demonstrated in invertebrates (2,16).

Memory can also be categorized based on its duration and concurrent molecular aspects. The retention time of memory ranges from minutes to hours for short-term memory, up to days to even a lifetime for long-term memory (2). The molecular requirements for each of these different types of memory have been found to be highly conserved, unifying the classification of memory across vertebrates and invertebrates. In this regard, short-term memory relies on immediate molecular actions, intermediary memory requires translation, and long-term memory additionally depends on *de novo* transcription (2).

While similar types of learning behaviors are observed across species, individual learning paradigms are designed based on each organism's innate behavior, or their evolutionarily determined responsiveness to environmental stimuli (17,18). Despite this diversity, it has become clear that associative and non-associative learning, short-term, intermediary and long-term memory rely on evolutionarily ancient mechanisms (8). Therefore, we will use this classification as a framework to discuss and compare the molecular basis of learning and memory examples.

2.2. Conservation of molecular mechanisms in learning and memory

Many molecular pathways have been implicated in the acquisition, storage and retrieval of memory and are extensively reviewed elsewhere (e.g. 19,20). Here, we briefly cover mechanisms for synaptic plasticity and experience-dependent gene transcription, before elaborating on the role of neuromodulators and their effects on these processes.

2.2.1. Mechanisms of synaptic plasticity

Experience-dependent alteration of synaptic strength, coined synaptic plasticity, is widely considered a primary mechanism for learning and memory. Synaptic plasticity may enhance (long-term potentiation, LTP) or depress (long-term depression, LTD) synaptic strength by modifications at either side of the synapse. Initial research bias towards a presynaptic model in invertebrates and a postsynaptic model in vertebrate studies led to the idea that different synaptic mechanisms underpin learning in vertebrates and invertebrates (21). Mounting evidence over the last years, however, largely resolved this bias indicating that the cellular basis of synaptic plasticity is evolutionarily well conserved (21).

Presynaptic plasticity, typically involving an increase or decrease of neurotransmitter release, was first established as a mechanism for non-associative learning in *Aplysia* and crayfish, regulated by cyclic adenosine monophosphate (cAMP) / protein kinase A (PKA) and protein kinase C (PKC) pathways (22–24). Associative learning was also found to rely on presynaptic cAMP/PKA and Ca^{2+} signaling controlling neurotransmitter release (25). Studies in *Drosophila melanogaster* further supported these findings by showing that cAMP/PKA signaling underlies associative olfactory learning (26). Similarly, in mice, presynaptic PKA signaling was found to mediate synaptic plasticity underlying contextual fear memory, an associative learning paradigm (27). The cAMP/PKA pathway has also been linked to presynaptically expressed LTP and LTD in several rodent studies, although associations with memory remain sparse (28).

The majority of research in vertebrates has focused on postsynaptic mechanisms for LTP and LTD in learning and memory, which generally involve changes in postsynaptic receptor numbers or signaling properties (19,29). Synaptic strengthening (LTP) or weakening (LTD) is mainly mediated by the activation of postsynaptic glutamate receptors of the N-methyl-D-aspartate (NMDA) and amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type receptor families (19,29). NMDA receptors (NMDARs) function as molecular coincidence detectors that are only activated upon depolarization of the postsynaptic membrane and concurrent release of glutamate from the presynaptic terminal. Once activated, NMDARs generate an influx of Ca^{2+} ions, activating a wide range of signaling pathways that facilitate LTP or LTD (19,30). While NMDAR-mediated LTP was first documented in vertebrates, mechanistically equivalent forms of plasticity have also been described in invertebrate nervous systems (21,31). Invertebrate NMDAR-mediated LTP was first discovered in *Aplysia*, revealing that both presynaptic activation of the PKA pathway and postsynaptic NMDAR-dependent influx of Ca^{2+} ions are required for synaptic plasticity and behavioral conditioning (32). Since then, NMDAR-dependent LTP has been reported in multiple invertebrate models

(33–36). Finally, trafficking of glutamatergic AMPARs on the postsynaptic membrane is a hallmark of NMDAR-dependent plasticity affecting synaptic strength, and considered crucial for learning in both invertebrate and vertebrate animals (37–39).

2.2.2. Experience-dependent transcription and long-term memory

While transient changes in existing proteins can alter synaptic strength and hereby encode short-term memory, the formation of long-term memory requires *de novo* protein synthesis (40). This has been demonstrated by the administration of inhibitors of transcription and translation during memory training, which attenuates long-term memory but leaves short-term memory unaffected in a wide range of vertebrate and invertebrate species (40). A central molecular player that regulates this transcriptional response is the cAMP response element-binding protein (CREB), a transcription factor crucial for long-term memory in many animal models (19,20,40). Upon activation, CREB regulates a wide set of downstream target genes, encompassing several synaptic and neuronal genes that are thought to mediate the structural and functional changes required for long-term memory (20). These include other transcription factors, histone deacetylases, synaptotagmins and shank proteins amongst many other targets (41–44). In *C. elegans*, CREB-activated genes have been identified in positive and negative long-term associative memory paradigms by means of RNA-Seq (44,45). A comparative analysis between these studies uncovered a core set of long-term memory genes regulated by CREB, which is recruited independent of the memory paradigm (45). Other CREB target genes only showed altered expression in response to either positive or aversive long-term memory training and may thus contribute to paradigm-specific neuronal changes. The majority of long-term memory genes appears to be indirectly regulated by CREB (44), and gene activation was found to occur in several waves after training (45). Many of the CREB-regulated genes identified in *C. elegans* are also known to function in neuronal processes, including memory, in mammals.

3. Neuromodulatory pathways in invertebrate learning circuits

Over the last decades many amine and neuropeptide neuromodulators have been implicated in learning and memory (4,7). These small signaling molecules, when binding to metabotropic receptors, activate biochemical signaling pathways that can alter the properties of single synapses, neurons or circuits by modifying neuronal dynamics, excitability or synaptic efficiency (46,47). Yet, our understanding of how neuromodulators influence learning and memory circuits is limited. Given their importance in the regulation of behavioral plasticity, many biogenic amines and neuropeptides are highly conserved across vertebrates and invertebrates (e.g. 5). This conservation, together with the relatively compact size of invertebrate nervous systems and the implementation of novel research tools for studying

neuromodulation, has made invertebrate learning circuits attractive models to dissect neuromodulator functions in learning and memory. Here, we provide a concise overview of aminergic and peptidergic pathways that affect learning and memory, and discuss findings on their mode-of-action in invertebrate learning circuits (summarized in Table 1).

3.1. Complexity of neuromodulatory pathways involved in learning and memory

3.1.1. Diversity of neuromodulators

A first indication for the role of monoamines in learning stems from studies on the gill-withdrawal reflex in *Aplysia*. This work showed that a single sensitizing stimulus to the tail triggers the release of serotonin from modulatory neurons onto the presynaptic terminals of a sensory neuron to increase its synaptic output and ultimately the behavioral response (48). Since then, several monoamines, including serotonin, dopamine, octopamine and noradrenaline, have been found to regulate learning (3,4,49). Neuropeptide signaling represents another route of neuromodulation in memory circuits, which was first demonstrated in *Drosophila* mutants for the neuropeptide-encoding gene *amnesiac*. These mutants are unable to consolidate aversive memories (50). Neuropeptides form an ancient and diverse class of secreted messengers that are increasingly recognized as modulators of learning circuits in vertebrates and invertebrates (7,51). Their storage, release mechanisms and temporal actions differ from monoamines and other classical neurotransmitters (47). Monoamines are packaged into synaptic vesicles, released into the synaptic cleft, as well as into dense core vesicles that can be released extrasynaptically. Biogenic amines can signal onto their postsynaptic partner on fast ms timescale and their effects are regulated by specific uptake and degradation mechanisms (52). In contrast, neuropeptides are mainly released extrasynaptically, from dense core vesicles, which have been found to be scattered across the nerve terminal and dendrites. Neuropeptides can mediate both local and long-range signaling. However, exocytosis of dense core vesicles is slow and generally requires high stimulation frequencies to raise calcium levels sufficient to trigger release. Unlike monoamines, no uptake mechanisms exist for neuropeptides. In addition, neuropeptides show large structural diversity, while the number of structurally distinct monoamines is limited. Both types of neuromodulators, however, can signal through a diverse range of GPCRs that often have distinct downstream effects (4,53). As such, neuromodulators can modify memory circuits through a wide range of actions, such as altering neurotransmitter release, modulating neuronal excitability, or stimulating neurogenesis (4,47). In addition, neuromodulators can interact through mutually antagonistic or synergistic relationships, further increasing the complexity of neuromodulatory effects in learning and memory (46).

3.1.2. Divergent and convergent neuromodulatory pathways

One theme emerging from studies of invertebrate learning circuits is the divergence of neuromodulator actions. One neuromodulator can target multiple receptors and circuits, providing mechanisms to orchestrate global behavioral states that may affect distinct types of learning or different aspects of memory. For example, a study in *C. elegans* demonstrated that release of the neuropeptide FLP-20 from primary mechanosensory neurons, triggered by aversive mechanical stimuli, induces a neuropeptidergic signaling cascade that exerts diverging effects on different circuits, such as locomotor arousal and sensitization of aversive chemosensation (54). For monoamines, which have limited structural diversity, distinct receptor targets have been found to mediate different modulatory actions on learning circuits. For example, D₁-like and D₂-like dopamine receptors, *dop-1* and *dop-3* respectively, both affect non-associative learning in *C. elegans*, but their actions are stimulus-dependent (55,56). While *dop-1* functions in mechanosensory neurons to modulate tap habituation depending on food availability, *dop-3* enhances avoidance behavior to repulsive odor signals independent of food availability (**Figure 2A**). These stimulus-dependent effects on non-associative learning are likely mediated by dopamine transmission from distinct dopaminergic neurons. (55,56). In *Drosophila*, dopamine released from the PPL1 dopaminergic neurons regulates different aspects of olfactory associative memory in the mushroom bodies (MB) in a receptor-dependent manner. Memory acquisition requires dDA1 receptor signaling and coincident Ca²⁺ and cAMP signaling, while signaling through another type of dopamine receptor, called DAMB (dopamine receptor in mushroom bodies), drives forgetting (57). These coordinated effects of dopamine could be important for removing labile memories and have been attributed to receptor-specific differences in intracellular signaling pathways and subcellular locations in the MBs (**Figure 2B**) (57,58). Another example is octopamine signaling driving different types of learning behavior, aversive and appetitive olfactory learning, in *Drosophila* by acting on distinct brain regions. Octopamine signaling via the β -adrenergic-like receptor Oct β 1R in the MB affects aversive learning, while Oct β 1R signaling in the projection neurons mediates appetitive learning (59).

The divergent actions of specific neuromodulators targeting distinct circuits or receptors is paralleled by the occurrence of convergent neuromodulatory pathways, when multiple neuromodulators affect the same learning circuit. This may allow different neuromodulators to translate environmental complexity into an appropriate physiological response, hereby fine-tuning learning behaviors to the exact environmental and internal context. A prime example of convergent neuromodulatory pathways is provided by the *C. elegans* gustatory aversive learning circuit. The neuropeptides nematocin, myoinhibitory peptide (MIP), neuromedin U (NMU) and the monoamines dopamine and serotonin all modulate *C. elegans* gustatory

aversive learning, although the extent by which these modulators act on shared neuronal elements remains unknown (60–62). Similarly, different insulin-like peptides influence *C. elegans* pathogen avoidance learning (63,64). Evidence for converging neuromodulatory actions also exists in other invertebrate model systems. For example, a recent study in *Drosophila* demonstrated that both serotonin and dopamine play a role in mate-copying, a form of social learning (65).

3.1.3. Synaptic and extrasynaptic actions

Many invertebrate studies found neuromodulators to mediate synaptic plasticity, for instance, by influencing neurotransmitter release from presynaptic neurons. For example, in *C. elegans*, insulin-like peptides feedback from postsynaptic interneurons onto the primary salt-sensory neuron ASER to mediate gustatory aversive learning by modifying both the excitability and synaptic release of the salt sensor (66). Insulin-related peptides have also been shown to affect synaptic plasticity by eliciting changes in synaptic connectivity after taste aversion learning in the mollusk *Lymnaea stagnalis* (67). Similarly, another study in *Drosophila* revealed that dopaminergic neurons contribute to long-term synaptic plasticity underlying aversive olfactory learning as optogenetic activation of dopaminergic neurons upon simultaneous odor exposure induces LTD in distinct regions of the MB lobes (68).

Despite having clear effects on synaptic plasticity, it is becoming increasingly clear that signaling from aminergic and peptidergic neurons not only affects postsynaptic neurons, but can modulate other neurons in learning circuits as well. Indeed, ample evidence suggests that neuromodulators can operate on broad spatial scales by communicating between neurons that are not synaptically connected. Mapping the putative monoamine connections and a subset of neuropeptide connections onto the *C. elegans* and *Platynereis dumerilii* synaptic connectomes suggests that a considerable portion of neuromodulatory signaling occurs extrasynaptically, i.e. between neurons that do not have synaptic connections (69–71). Several *C. elegans* neuropeptides are known to signal extrasynaptically in learning and memory circuits, including FLP-20 neuropeptides in sensitization (54), nematocin, MIP and NMU neuropeptides in gustatory aversive learning (60–62) and INS-6 in aversive olfactory learning (64). The broad spatial scales on which neuromodulators can act, together with their structural and functional diversity, vastly increases the potential for neuromodulation in learning and memory circuits, but also challenges functional studies of aminergic and peptidergic pathways in learning.

3.2. Neuromodulation of learning and memory at the circuit level

3.2.1. Approaches for studying neuromodulation in learning circuits

Technological developments to record and manipulate neural circuit activity, including optogenetics and *in vivo* calcium imaging, advanced research into the mechanisms underlying learning and memory (29,68,72). Many of these techniques are now also being employed to elucidate the actions of neuromodulators in learning circuits. Several invertebrate model systems offer the advantage of a compact nervous system in which synaptic and neuromodulatory connectivity has been well characterized, facilitating functional studies of neuromodulators in learning circuits. The complete neuron morphologies and synaptic connections of key memory centers (73,74) or even entire nervous systems (71,75,76) have been reconstructed using electron microscopy for *Drosophila*, *C. elegans* and *Platynereis*. In addition, methods for transcriptome profiling of single cells and cell types have been applied to chart the complement of neuromodulators across neurons in learning circuits (77–80). This allows these neuromodulatory connections to be mapped onto the synaptic connectome, which has highlighted the notion that neuromodulators mainly signal extrasynaptically while also forming a tractable framework for comprehensive analysis of neuromodulator action within learning circuits (69–71,81).

A growing body of work in *C. elegans* and *Drosophila* is employing neural recording and activity manipulation to investigate neuromodulator actions in various forms of learning and memory. Especially light- and temperature-sensitive proteins (e.g. ChR2 or TrpA1) for precise control of neurotransmission are finding application to probe the temporal contribution of aminergic and peptidergic neurons in learning processes (62,82,83). These studies highlight defined roles of these neurons in specific learning or memory phases. For example, timed artificial stimulation of neurons producing the *Drosophila* ortholog of the mammalian neuropeptide Y (NPY), called neuropeptide F (NPF), strengthens the retrieval of long-term appetitive olfactory memory in adult *Drosophila* (82), while attenuating the formation of short-term appetitive olfactory memory in fly larvae by modulating the sugar reward that is paired to odor cues (84). The efficiency of neuromodulator release triggered by optogenetic or thermogenetic stimulation, however, represents a possible drawback of this technique. Alternative methods to directly manipulate the levels of second messengers driving vesicle exocytosis are being developed to overcome this limitation (85). Conversely, optogenetic reagents can be used to silence neuropeptidergic neurons during discrete periods of learning and memory processing. For instance, temporal inhibition of the *C. elegans* neuron pair expressing the NMU-ortholog CAPA-1 uncovered this neuropeptide to be specifically required for the retrieval of learned salt aversion (62). Likewise, the temperature-sensitive *Shibire*^{ts} channel and the chemogenetic silencer HisCl1 are used in *Drosophila* and *C. elegans*,

respectively, to delineate neuronal populations underlying either the formation or retrieval of specific memories (86–88). Altogether, these studies highlight that discrete neural populations within neuromodulatory signaling axes can have precise temporal contributions to distinct forms of behavioral plasticity. However, further work is needed to characterize the exact context- and temporal-specific actions of neuromodulators at a cellular level.

One particularly exciting prospect is the implementation of new tools to visualize the release and subsequent GPCR activation of these neuromodulators. For instance, novel neuropeptide vesicle exocytosis reporters that relay changes in acidity between the dense core vesicle lumen and the extracellular space were recently used to visualize neuropeptide release in *Drosophila* (89) and *C. elegans* (90). GPCR-based ligand sensors to visualize neuromodulator action at a cellular resolution have also been developed by various research groups (91,92). While they have yet to be applied in the study of learning circuits, these tools are certain to improve our understanding of neuromodulatory signaling within these circuits.

3.2.2. Modulation of sensory processing

At the circuit level, neuromodulators have often been shown to affect learning circuits by regulating sensory processing, hereby altering the ability of sensory circuits to respond to environmental cues (93). Pioneering studies in *Aplysia* first revealed this mechanism by studying sensitization (11,94). Facilitation of the gill-withdrawal reflex after exposure to a noxious stimulus is mediated by serotonin release. Serotonin acts on sensory neurons to enhance neurotransmitter release and synaptic strength (20). Persistent facilitation also stimulates serotonin release, which subsequently induces long-term changes in synaptic strength through CREB-mediated transcription in sensory neurons (20,93). Conversely, the same molecular mechanisms mediate habituation after electrical stimulation of single sensory neurons by decreasing neurotransmitter release and concurrent synaptic depression (24).

Many neuromodulatory systems have been found to modulate sensory processing in invertebrate learning circuits by acting on sensory neurons, although their precise effects often remain unknown. For example, the *C. elegans* nematocin receptor NTR-1 is required in the primary salt-sensory neuron ASEL, for nematocin - a vasopressin/oxytocin-related peptide - to promote gustatory aversive learning (60). Similarly, insulin-like peptides signal from the AIA interneurons to both AWC and ASER sensory neurons to mediate *C. elegans* aversive olfactory learning and gustatory aversive learning, respectively (95,96). In addition to direct actions on sensory neurons, neuromodulatory systems can affect sensory processing downstream, for example, by acting at the interneuron level. This is illustrated by several examples in *C. elegans* and insects. *C. elegans* pathogen avoidance learning requires serotonergic signaling from chemosensory neurons to serotonin-gated chloride channels on

downstream interneurons (97). Dopaminergic neurons in the *Drosophila* MB also modulate synaptic transmission along Kenyon cell axons to convey odor signal output during olfactory learning (98). Octopamine plays an important role in appetitive olfactory learning in the honeybee *Apis mellifera*. Odor detection stimulates octopaminergic neurons in the antennal lobe to modulate output activity of downstream project neurons (99).

3.2.3. Temporal requirements of neuromodulators

Work in various invertebrate model systems highlights neuromodulators to influence learning and memory circuits across diverse temporal scales. In *C. elegans* NMU-like neuropeptides are involved in gustatory aversive learning that specifically mediate the retrieval of learned salt avoidance, but are not required for the acquisition of this response (**Figure 3A**) (62). Temporally defined effects on learning and memory have also been observed for other neuropeptides, such as NPY/NPF. In *Drosophila* larvae, NPF neurons modulate sugar reward during appetitive olfactory learning. Artificial activation of NPF neurons affects appetitive olfactory learning specifically during memory acquisition but not during retrieval (**Figure 3B**) (84).

Besides affecting specific phases of learning and memory, neuromodulators can also specifically regulate different temporal types of memory. Temporal control of octopamine signaling in *Drosophila* shows that this monoamine, which typically signals reward in insects, is specifically required for the formation of short-term olfactory appetitive memory but not for its long-term counterpart (100). Dopamine signaling also plays a role in olfactory appetitive memory in *Drosophila*. However, distinct dopaminergic neuronal subsets within the protocerebral anterior medial (PAM) cluster induce short-term and long-term memories (101). Examples of neuropeptides regulating specific temporal forms of memory in *Drosophila* are amnesiac and insulin. Amnesiac signaling in the MB regulates the formation of middle-term and long-term aversive olfactory memories (102), while insulin is acutely required for long-term memory formation, as its disruption in the ellipsoid body specifically impairs long-term memory (103).

3.3. Neuromodulators as regulators of context-dependent learning in invertebrates

Learning evolved as an adaptive trait to suit an organism's needs. Hence, the ability to learn is regulated by internal and external context. Various environmental factors have been shown to affect learning in invertebrates, including food availability, the presence of conspecifics, temperature, oxygen availability, environmental light and a variety of stressors, such as oxidative stress (104), predator-induced stress (105), and chemicals e.g. insecticides, caffeine and ethanol (106–108). Neuromodulators have emerged as important regulators of context-dependent learning and memory, yet the multitude of reported context-dependent changes in

learning circuits contrasts with the scarce knowledge of the underlying mechanisms. A few notable exceptions that have been investigated in more depth are metabolic, social and reproductive contexts.

3.3.1. Food context

Satiation and metabolic state are well-studied internal states that alter neural plasticity (109). As such, restricting food or water intake generally enhances acquisition and retrieval of learned behavior (82,109–111). Many food-dependent behaviors are mediated by evolutionarily conserved neuromodulators, including serotonin, dopamine, and NPY/NPF amongst others (100,112,113). In *Drosophila* and *C. elegans*, serotonin enhances associative learning under dietary restriction (109,114,115). The role of dopamine signaling in food-dependent learning depends on the specific context. While sensitization is suppressed by dopamine signaling in well-fed *C. elegans* (55,116), dopaminergic neurons in the hungry fly brain enhance appetitive associative learning (82).

Often, the actions of several different neuromodulators mediate the effects of metabolic state on learning ability. In *Drosophila*, NPF acts in concert with dopamine to enhance olfactory associative learning upon prolonged food deprivation (82). The combined effect of NPF and dopamine in the MB is thought to function as a motivational switch that only allows robust memory retrieval when animals are starved. Thereby, these neuromodulators effectively adjust the output of appetitive memory to the context in which they act adaptively (82). In *C. elegans*, dopamine and sNPF-like neuropeptide signaling both enhance olfactory non-associative learning after food-deprivation, albeit not necessarily in direct interaction (55,117).

3.3.2. Social context

Besides food-context, social context has been widely shown to influence learning in diverse animals. Crowding reduces associative learning in *C. elegans* and *L. stagnalis* (118–120). In *C. elegans*, this phenomenon is initiated by pheromone detection and involves insulin-like peptide signaling as well as signaling through the *C. elegans* homolog of the elevenin neuropeptide SNET-1 (**Figure 4A**) (118,119). Social context also affects learning in the honeybee *A. mellifera*. Queen mandibular pheromone reduces associative learning in worker bees by suppressing juvenile hormone synthesis. Juvenile hormone in turn affects dopamine and octopamine receptor gene expression in the MB (**Figure 4B**) (121). In addition, in honeybees stress-related changes in learning performance are induced by the alarm pheromone isopentyl acetate (122). These changes can be mimicked by the administration of allatostatin neuropeptides, suggesting that allatostatins may modulate learning performance in response to alarm signals (122).

3.3.3. Reproductive context

Context dependency of learning is not limited to the relatively fast changes that can occur in satiation and metabolic state, or social context, but is also affected by physiological differences between sexes. Hence, it is not surprising to find sex and reproductive state-dependent differences in neuromodulation of learning, which can often be explained by adaptive fitness advantages. In this regard, *C. elegans* males show decreased salt aversion as compared to hermaphrodites after paired exposure to salt and food deprivation, which is thought to result from decreased IGF-1 signaling in male worms (123). Moreover, *C. elegans* males, but not hermaphrodites, fail to acquire learned salt aversion when hermaphrodite worms are present during conditioning (124). This behavior, in which males seem to prioritize mates over food, is mediated by release of the PDF ortholog, PDF-1, from male-specific neurons (124).

Sexually dimorphic learning behavior has also been studied in *Drosophila*, where males can both associatively and non-associatively learn to suppress courtship towards females after being rejected by previously mated females. This complex behavior is known as courtship conditioning (125). When being rejected by a mated female, octopaminergic and dopaminergic neurons signal through male-specific projections on the mushroom body α/β - and γ -lobes, which results in decreased courtship behavior. Besides several other mechanisms that govern courtship conditioning, rejection increases male sensitivity to 11-*cis*-vacacenyl acetate (cVA), a male-produced sex pheromone that is transferred in low quantities from males to females during mating (125,126). When a mating attempt fails, dopaminergic neurons convey a learning signal to the MBs, inducing a permanent change in cVA processing which allows males to discriminate mated females from unmated females (125).

4. Bridging neuromodulation in invertebrate and vertebrate learning circuits

Several of the molecular players and mechanisms involved in neuromodulation of invertebrate learning circuits seem to play a role in vertebrate memory circuits as well. Indeed, aside from a clear structural conservation, many of the fundamental signaling principles of aminergic and peptidergic systems are conserved across the major animal phyla (127). Ample work in rodent models highlights the effects of monoamines, such as dopamine and serotonin, on synaptic plasticity and learning (reviewed in 4), similar to the functions of these neuromodulators in invertebrate learning circuits. For example, signaling from the dopamine D1 receptor modulates spatial learning and memory in the prefrontal cortex by driving extensive synaptic restructuring in rats (128). Neuropeptides affect synaptic plasticity and learning in vertebrates as well. A notable example is oxytocin, which modulates fear learning associated with long-term synaptic plasticity in mice (129). Oxytocin/vasopressin-like neuropeptides have also been

shown to regulate learning in *C. elegans* (60). Other neuropeptides that are involved in vertebrate and invertebrate learning include NPY/NPF, neuromedin U and tachykinin (9,62,82,130,131). These neuropeptide families may be ancient regulators of learning circuits, although it remains to be understood if the underlying neuromodulatory mechanisms are conserved between vertebrates and invertebrates.

Work in vertebrate models also highlights specific temporal contributions of neuromodulators within functionally distinct phases of memory processing, as was observed in several invertebrate models for octopamine, dopamine and neuropeptides. In mice, for example, chemogenetic silencing of serotonergic neurons shows that this neuromodulator is specifically required for the acquisition and consolidation of object memory, while not affecting its retrieval (132). Likewise, distinct neuromodulatory effects on the temporal aspects of memory retention are apparent in vertebrate models. In rats, the neuropeptide cortistatin is crucial for the formation of long-term memory when assessing passive avoidance, whereas it does not influence short-term memory (133).

While we are only beginning to understand the mechanisms and neuromodulatory systems that mediate context-dependent learning, a role for diverse neuromodulatory systems has also emerged from vertebrate studies. Vertebrates rely heavily on cues from conspecifics to guide behavior. As such, social context profoundly affects vertebrate learning. For example, alarm pheromones are known to enhance fear conditioning in rats, which is mediated by norepinephrine signaling (134). Zebra finch song learning is another classic example where social context affects learning in vertebrates. Socially interacting zebra finches perform better at song learning than those that are kept from interacting, an effect mediated by dopamine release upon social interactions (135,136). Other neuromodulators, including norepinephrine and avian oxytocin-like signaling, are likely to enhance learning exclusively in male zebra finches upon social interactions as well (135,137). Taken together, these studies suggest a common ground between neuromodulators and their effects on learning in both vertebrates and invertebrates. Yet, further research in different model systems is needed to gain a more detailed understanding and comparison of the underlying mechanisms.

5. Conclusion

The examples illustrated here establish a clear role for neuropeptides and monoamines as modulators of learning and memory. The advance of research tools to record and manipulate neural activity across a wide range of organisms has started uncovering the diffuse actions of neuromodulatory pathways that affect learning circuits. Many neuromodulators act across spatiotemporal scales or in response to internal and external cues, such as food and social context. Furthermore, neuromodulatory pathways often interact with each other through

mutually antagonistic or synergistic relationships. It is becoming increasingly clear that concerted actions of several neuromodulators govern specific types of learning and memory. It will be interesting to further unravel the functions and plasticity of these neuromodulatory networks, and to gain deeper insight into the mechanisms by which neuromodulators regulate learning and memory.

Throughout the past decades, invertebrate model systems have been instrumental in uncovering the molecular pathways that underlie synaptic plasticity and in clarifying the modes of action of neuromodulators in learning circuits. Their tractable and relatively compact nervous systems have allowed the study of neuromodulatory signaling axes at a cellular resolution. We foresee these features to also support the implementation of novel methods, such as those for stimulating and monitoring neuromodulator release and actions. This promises to rapidly advance our understanding of the precise actions of neuromodulators within learning circuits. Given the structural and functional similarities of neuromodulatory systems across vertebrate and invertebrate models, invertebrate studies are bound to help further uncover neuromodulator actions in learning and memory. Integrating molecular and functional knowledge on neuromodulation of learning circuits from different invertebrate and vertebrate models will also shed light on the evolution of neuromodulatory functions and learning behavior.

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Table 1: Overview of monoamines and neuropeptides involved in learning and memory discussed in section 3.

Neuromodulator	Memory type	Species ^A	Paradigm	References
Serotonin	Sensitization	<i>A. californica</i>	Electrical sensitization	(11,94)
	Classical conditioning	<i>C. elegans</i>	Appetitive gustatory learning	(107)
			Gustatory aversive learning	(60)
			Pathogen avoidance learning	(97)
		<i>D. melanogaster</i>	Appetitive olfactory learning	(115)
	Social memory	<i>D. melanogaster</i>	Mate-copying	(65)
Dopamine	Classical conditioning	<i>A. mellifera</i>	Aversive olfactory learning	(121)
		<i>C. elegans</i>	Appetitive gustatory learning	(114)
			Aversive gustatory learning	(60)
		<i>D. melanogaster</i>	Appetitive olfactory learning	(82,101,109)
			Aversive olfactory learning	(57,68,115)
			Courtship conditioning	(125)
	Habituation	<i>C. elegans</i>	Tap habituation	(55,116)
		<i>D. melanogaster</i>	Courtship conditioning	(125)
	Sensitization	<i>C. elegans</i>	Olfactory avoidance	(56)
	Social memory	<i>D. melanogaster</i>	Mate-copying	(65)
Octopamine	Classical conditioning	<i>A. mellifera</i>	Appetitive olfactory learning	(99)
			Aversive olfactory learning	(121)
		<i>D. melanogaster</i>	Appetitive olfactory learning	(59,101)
			Aversive olfactory learning	(59)
			Courtship conditioning	(126)
	Habituation	<i>D. melanogaster</i>	Courtship conditioning	(126)
Allatostatin	Classical conditioning	<i>A. mellifera</i>	Appetitive olfactory learning	(122)
Amnesiac	Classical conditioning	<i>D. melanogaster</i>	Aversive olfactory learning	(102)
Elevenin	Classical conditioning	<i>C. elegans</i>	Benzaldehyde-starvation learning	(119)
FLP-20 RFamide	Sensitization	<i>C. elegans</i>	Sensitized responses to tap and aversive chemicals	(54)
Insulin-like peptides	Classical conditioning	<i>C. elegans</i>	Aversive olfactory learning	(64,95)
			Aversive gustatory learning	(66,96)
			Appetitive gustatory learning	(123)
			Pathogen avoidance learning	(63,64,118)
		<i>L. stagnalis</i>	Taste aversion learning	(67)

		<i>D. melanogaster</i>	Aversive olfactory learning	(103)
Myoinhibitory peptide	Classical conditioning	<i>C. elegans</i>	Appetitive gustatory learning	(61)
Nematocin	Classical conditioning	<i>C. elegans</i>	Appetitive gustatory learning	(60)
Neuromedin U	Classical conditioning	<i>C. elegans</i>	Appetitive gustatory learning	(62)
Neuropeptide F	Classical conditioning	<i>D. melanogaster</i>	Appetitive olfactory learning	(82,84)
Pigment dispersing factor	Classical conditioning	<i>C. elegans</i>	Appetitive gustatory learning	(124)
Short neuropeptide F	Habituation	<i>C. elegans</i>	Olfactory aversive learning	(117)

^A*A. californica*, *Aplysia californica*; *A. mellifera*, *Apis mellifera*; *C. elegans*, *Caenorhabditis elegans*; *D. melanogaster*, *Drosophila melanogaster*; *L. stagnalis*, *Lymnea stagnalis*

Figure legends

Figure 1: In vertebrates, memory is traditionally structured based on content. Explicit memory entails the conscious recollection of data, including facts (semantic memory) and events (episodic memory). Conversely, implicit memory encompasses nonconscious abilities and previous experiences that can be recalled without awareness. Implicit memory covers priming, procedural memory, associative and non-associative learning. Associative and non-associative learning are further categorized in classical conditioning and operant conditioning, and sensitization and habituation, respectively. While explicit memory is almost exclusively studied in vertebrates, conceptually and behaviorally similar forms of implicit memory have been described in molecular and cellular detail in several invertebrate models.

Figure 2: Neuromodulators can exert receptor-specific effects on learning circuits. (A) In *C. elegans*, distinct dopamine-like receptors affect non-associative learning in a stimulus-dependent manner. Enhancement of 2-nonanone avoidance, sensed by AWB neurons, requires dopaminergic signaling via DOP-3 in RIC interneurons. In addition, release of dopamine from dopaminergic neurons (DAN), including the CEP neurons, stimulates DOP-1 in touch receptor neurons (TRN), such as ALM, to modulate the rate of mechanosensory habituation in the presence of food during repeated mechanosensory stimulation of the worm's head (anterior tap). (B) Dopaminergic signaling in *Drosophila* affects both acquisition and forgetting of olfactory memories after olfactory aversive learning through dDA1 and DAMB receptor signaling, respectively. AL, antennal lobe; DAN, dopamine neurons; MB, mushroom bodies; DA, dopamine.

Figure 3: Temporal requirements of neuromodulators in learning and memory circuits. (A) In *C. elegans*, CAPA-1 Neuromedin U (NMU)-like neuropeptides mediate the retrieval of learned salt avoidance during gustatory aversive learning by activating their receptor NMUR-1 in AFD sensory neurons. (B) Conversely, in *Drosophila* larvae release of neuropeptide F (NPF) from dNPF neurons in the larval brain specifically mediates the acquisition phase during appetitive olfactory learning. dNPF-DL1, dNPF-dorsolateral cell 1; dNPF-DL2, dNPF-dorsolateral cell 2; dNPF-sog, dNPF-subesophageal ganglion.

Figure 4: Neuromodulators are important regulators of context-dependent learning. (A) In *C. elegans*, expression of the elevenin-like neuropeptide SNET-1, a negative regulator of olfactory aversive learning, is dependent on the population density. In addition, population density influences pathogen avoidance learning through the action of two insulin-like peptides (INS). (B) Queen mandibular pheromone (QMP) reduces aversive learning in worker bees by suppressing juvenile hormone synthesis, which in turn modulates expression of the octopamine receptor gene (*Amoa1*) and dopamine receptor gene (*Amdop1*). CC, corpora cardiaca; AL, antennal lobe; MB, mushroom bodies.

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