Review

Feeding behavior of *Aplysia*: A model system for comparing cellular mechanisms of classical and operant conditioning

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Feeding behavior of *Aplysia* provides an excellent model system for analyzing and comparing mechanisms underlying appetitive classical conditioning and reward operant conditioning. Behavioral protocols have been developed for both forms of associative learning, both of which increase the occurrence of biting following training. Because the neural circuitry that mediates the behavior is well characterized and amenable to detailed cellular analyses, substantial progress has been made toward a comparative analysis of the cellular mechanisms underlying these two forms of associative learning. Both forms of associative learning use the same reinforcement pathway (the esophageal nerve, En) and the same reinforcement transmitter (dopamine, DA). In addition, at least one cellular locus of plasticity (cell B51) is modified by both forms of associative learning. However, the two forms of associative learning have opposite effects on B51. Classical conditioning decreases the excitability of B51, whereas operant conditioning increases the excitability of B51. Thus, the approach of using two forms of associative learning to modify a single behavior, which is mediated by an analytically tractable neural circuit, is revealing similarities and differences in the mechanisms that underlie classical and operant conditioning.

In their habitat, animals face a constantly changing environment. It is advantageous if the more salient aspects of the environment can be predicted. Operant and classical conditioning constitute important processes by which predictive memory is acquired. In classical conditioning, an initially neutral event (conditioned stimulus, CS) that precedes an important event (unconditioned stimulus, US) may come to function as a predictor of the US. Following the association between CS and US, the animal will produce anticipatory behavior (conditioned response, CR) when presented with the CS (Pavlov 1927). In operant conditioning, an animal learns to anticipate an important event (reward or punishment) that consistently occurs as a result of the animal's own behavior (the operant). By forming an association between the operant and reinforcement, the animal learns the consequences of its behavior and changes the frequency of the operant accordingly (Thorndike 1911; Skinner 1938). Thus, these forms of learning constitute important adaptive mechanisms for behavior.

A fundamental problem in neuroscience is to understand events occurring within individual neurons and within networks that contribute to learning and memory. For example, what cellular processes detect the coincidence between stimuli during classical conditioning, or between behavior and consequences during operant conditioning? What are the neural substrates that store this information? How are subsequent behaviors modified? Although these forms of associative learning can be distinguished procedurally, are the mechanisms underlying operant and classical conditioning similar or different? And if so, at what organizational level?

The consummatory phase of feeding behavior in *Aplysia* exhibits several features that make it an attractive model system with which to study associative learning. The behavior can be

modified by both classical and operant conditioning (Susswein and Schwartz 1983; Susswein et al. 1986; Colwill et al. 1997; Botzer et al. 1998; Lechner et al. 2000a; Brembs et al. 2002; Katzoff et al. 2002; Lorenzetti et al. 2006b). The behavior occurs in an all-or-nothing fashion and is therefore easily quantified (Brembs et al. 2002). In addition, the neural circuitry underlying the behavior is well characterized and amenable to detailed cellular and molecular analysis (for recent reviews, see Elliott and Susswein 2002; Cropper et al. 2004). This review summarizes recent advances in a comparative analysis of the cellular mechanisms that underlie appetitive classical conditioning and reward operant conditioning of feeding behavior, specifically biting. Both forms of associative learning share a common reinforcement pathway (the esophageal nerve, En) and both use dopamine (DA) as a reinforcement transmitter. Moreover, both forms of associative learning share at least one cellular locus of plasticity, cell B51, which is believed to make a critical contribution to feeding behavior. However, the two forms of associative learning have opposite effects on B51. Classical conditioning decreases the excitability of B51, whereas operant conditioning increases the excitability of B51. Thus, these two forms of associative learning appear to differ at the cellular level.

Feeding behavior, motor patterns, and the feeding neural network

Feeding behavior and its neural network

Consummatory feeding in *Aplysia* consists of a series of rhythmic movements involving the peri-oral structures and the foregut, including the mouth (lips and jaws), buccal mass (odontophore and radula), and esophagus (for review, see Kupfermann 1974; Kandel 1979; Elliott and Susswein 2002; Cropper et al. 2004). Ingestion draws food into the mouth, through the buccal cavity and toward the esophagus. During ingestion, the odontophore, with its two radula halves (toothed grasping surfaces), is first rotated forward toward the mouth (i.e., protraction) and the jaws

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open to accommodate the protracting odontophore. Initially, the two halves of the radula are separated during protraction, but before the peak of protraction, they begin to close and grasp the food. The radula remains closed as the odontophore retracts (backward rotation), which brings the food into the mouth and buccal cavity, and the jaws close as the odontophore retracts (Morton and Chiel 1993a) (for an example of ingestion, see the video clip included in the Supplemental material in Brembs et al. 2002). In addition to ingestion, the foregut produces rejection movements in response to inedible or distasteful objects taken into the buccal cavity (Morton and Chiel 1993a). During rejection, the two halves of the radula are closed as the odontophore protracts toward the mouth and they are open as the odontophore retracts, which ejects the inedible object from the buccal cavity. Thus, consummatory feeding behaviors can be described as having two phases: a protraction phase followed by a retraction phase. During ingestion the radula is closed during the retraction phase, whereas, during rejection, the radula is open during the retraction phase.

The neural circuitry that mediates consummatory feeding behavior is located primarily in the cerebral and buccal ganglia (for recent reviews, see Elliott and Susswein 2002; Cropper et al. 2004). As a result of work in several laboratories, this circuitry is beginning to be understood. Figure 1 illustrates several elements of the neural circuitry for feeding behavior that have been well characterized. For purposes of this discussion, this circuit can be loosely organized into a hierarchy. The cerebral ganglia contain sensory afferents that mediate tactile information (e.g., cerebral mechanoafferents [CM]; Rosen et al. 1979, 1982) and the cerebral ganglia receive chemosensory information (Xin et al. 1995) from the lips and other regions of the head. Mechanosensory and chemosensory inputs converge onto cerebral-buccal interneurons (e.g., CBI-2), some of which can elicit neural activity for feeding behavior. These higher-order neurons are therefore referred to as command-like neurons (Rosen et al. 1991; Morgan et al. 2000; Sanchez et al. 2000; Jing and Weiss 2001; Sanchez and Kirk 2001; Morgan et al. 2002; Sanchez and Kirk 2002; Jing and Weiss 2005; for review, see Kupfermann and Weiss 2001). The motor activity that controls the rhythmic movements of the odontophore and radula is generated by a central pattern generator (CPG) in the buccal ganglia (e.g., Susswein and Byrne 1988; Church and Lloyd 1994; Horn and Kupfermann 2002).

Motor patterns and neural activity underlying feeding movements

Many previous studies have utilized a variety of intact, semi-intact, and reduced preparations to relate specific patterns of neural activity to aspects of consummatory feeding behaviors. For example, Morton and Chiel (1993a) combined video recordings of consummatory feeding behaviors with extracellular recordings of buccal nerve activity in intact animals. Two patterns of neural activity (buccal motor patterns [BMPs]) were characterized, one recorded during ingestion and the other recorded during rejection. The two BMPs were distinguished by the timing of large-unit activity in the radula nerve (Rn) relative to the onset of activity in the buccal nerve 2 (n2). During ingestion, large-unit activity in the two nerves primarily overlapped (see insert in Fig. 1), whereas, during rejection, large-unit activity in Rn preceded that in n2. Moreover, extracellular recordings from behaving animals were later associated with intracellular recordings from identified neurons in a reduced preparation (Morton and Chiel 1993b; see also Church and Lloyd 1994; Orekhova et al. 2001; Horn et al. 2004; Jing et al. 2004; Ye et al. 2006). The large-unit activity that was recorded extracellularly in Rn corresponded to activity in closer motor neurons B8a/b and one of the large units that was recorded extracellularly in n2 corresponded to activity in retractor motor neuron B10. Thus, during ingestion BMPs,

Figure 1. Several elements of the neural circuit for feeding behavior. Sensory neurons in the cerebral ganglion (i.e., cerebral mechanoafferents, CM) mediate tactile stimuli from the lips. A subclass of CM cells (i.e., interganglionic cerebral buccal mechanoafferents, ICBM) projects to the buccal ganglion. Chemosensory cells are thought to have cell bodies in the periphery and project to neurons in the cerebral ganglia. Cerebral-to-buccal interneurons (CBI) receive mechanosensory and chemosensory input and project to the central pattern generator (CPG) in the buccal ganglia. Some CBIs (e.g., CBI-2) can evoke patterned activity in the CPG and are therefore referred to as command-like neurons. Some of the neurons in the CPG can be assigned functional roles. For example, B31/32 and B63 play important roles in initiating buccal motor patterns (BMPs) and maintaining the protraction phase of a BMP. B64 plays an important role maintaining the retraction phase. B4/5, B34, and B51 play important roles in switching the activity of the CPG between and rejection- and ingestion-like BMPs. The expression of activity for ingestion or rejection is determined by the phase relationship of activity in closure motor neurons (e.g., B8) and the protraction/retraction cycle. Pattern-switching neurons shift the closure activity either toward the protraction phase to produce rejection-like BMPs (e.g., B4/5) or toward the retraction phase to produce ingestion-like BMPs (e.g., B51). Patterned activity is terminated, in part, by activity in neuron B52. The circuitry for feeding behavior is under the control of a number of modulatory neurons, such as the serotonergic metacerebral cell (MCC). Note that this diagram is not a comprehensive description of available data on identified neurons and synaptic connections involved in the control, expression, and modulation of feeding behavior. The inset illustrates two BMPs with the protraction, closure, and retraction activity monitored from peripheral nerves I2, Rn, and n2, respectively. One of the BMPs is an example of an ingestion-like BMP (i-BMP) and the other is an example of a rejection-like BMP (r-BMP). A BMP was classified as ingestion-like if 50% or more of the large-unit activity in Rn, which represents activity in radula closure motor neurons, occurred after the end of the burst in the I2 nerve, which represents activity in radula protraction motor neurons. A BMP was classified as rejection-like if the large-unit activity in Rn did not extend beyond the burst in the I2 nerve. The gray boxes that are below the middle trace indicate the duration of large-unit activity in the Rn. (Modified with permission from The Society for Neuroscience © 2000, Lechner et al. 2000b.)

activity in closer and retractor motor neurons coincided (i.e., overlapping large-unit activity in Rn and n2), whereas, during rejection-like BMPs (r-BMPs), activity in closer motor neurons preceded the activity in retractor motor neurons. These studies provide a useful framework or set of criteria for evaluating the potential behavioral relevance of patterns of neural activity observed in more reduced preparations.

Reduced preparations of isolated ganglia retain the ability to express patterns of neural activity similar to those previously recorded in vivo (e.g., Susswein and Byrne 1988; Morton and Chiel 1993b; Nargeot et al. 1997, 1999a,b; Kabotyanski et al. 2000; Jing and Weiss 2001; Hurwitz et al. 2003). This feature has facilitated analysis of the role of specific cellular components of the buccal CPG (Fig. 1) in the generation of BMPs. For example, B51, a neuron pivotal for the production of ingestion-like BMPs (Nargeot et al. 1999a,b), exhibits a characteristic, sustained, all-or-nothing level of activity (plateau potential) (Plummer and Kirk 1990) during ingestion-like BMPs, but not during rejection BMPs (Nargeot et al. 1999a). Moreover, direct depolarization of B51 during a BMP enhances the production of ingestion-like BMPs, whereas hyperpolarization inhibits ingestion-like BMPs (Nargeot et al. 1999a).

Associative learning and feeding behavior

Appetitive classical conditioning

Lechner et al. (2000a) developed a training protocol for classical conditioning of biting in *Aplysia* that produced both short- and long-term memory. The procedure was similar to one developed earlier by Colwill et al. (1997). Conditioning was accomplished by delivering paired presentations of a conditioned stimulus (CS, tactile stimulation of the lips with a soft paintbrush) with an unconditioned stimulus (US, seaweed) (Fig. 2). The effects of conditioning were assessed by counting the number of bites elicited by CS test stimuli, which were delivered prior to training (pre-test) and 1 or 24 h after training (post-test). At both time points after training, paired training produced a significant increase in the number of bites (post-test minus pre-test) as compared to unpaired training (Fig. 3). These data indicate that feeding behavior in *Aplysia* can be classically conditioned and that the associative memory persists for at least 24 h. This finding provided the basis for subsequent cellular analyses (see below).

Identifying the reinforcement pathway

In the classical conditioning procedure, the animal is allowed to ingest the seaweed (i.e., the US). Thus, the US activates sensory pathways both from the lips and from the foregut. To analyze which of these pathways mediate the conditioning, the US presentation was manipulated to stimulate only the lip region (Lechner et al. 2000a). This stimulus (i.e., touching the lips with seaweed) reliably elicited bites (i.e., the UR). During training, animals received either paired or unpaired CS/US presentations in which seaweed (i.e., the US) was brought into contact with the lips, but the animal was not allowed to ingest the food. A separate group of animals received training in which they were allowed to ingest the food. Learning was evaluated 1 h after training. Only animals that received paired training and that were allowed to ingest the food displayed a significant increase in the number of bites. These data provide evidence that the sensory pathways originating from the lips of the animals do not support conditioning and suggest the US pathway originates in the foregut.

Schwarz and Susswein (1984, 1986) found that an intact esophageal nerve (En) was necessary for *Aplysia* to learn a food was inedible, which suggests En conveys information about the presence of food in the gut during ingestive behavior and may mediate reinforcement during associative learning. To provide

additional insights into the potential role of En as a reinforcement pathway, extracellular recordings of nerve activity were made in freely behaving *Aplysia* via chronically implanted extracellular hook-electrodes on the En (Brembs et al. 2002). Little nerve activity was observed during biting in the absence of food. In contrast, brief (~3 sec) bursts of high-frequency (~30 Hz) nerve activity were recorded in En during the ingestion of food. This observation suggests that the En conveys afferent information related to the US.

To examine the role of afferent pathways originating from the foregut in classical conditioning, the anterior branch of esophageal nerve (En₂) was surgically lesioned (Lechner et al. 2000a). Animals with bilateral lesions of the En₂ received paired or unpaired CS/US presentations and were compared with similarly trained sham operated animals. En₂ lesions did not hamper the ability of animals to bite and ingest small pieces of food (e.g., the US during conditioning). However, 1 h after training, only the sham operated animals demonstrated a pairing-specific increase in bites elicited by the test CS. These data suggest that the afferent pathways originating in the foregut play a key role in mediating the US in appetitive classical conditioning of feeding in *Aplysia*. Whether the fibers within En₂ themselves or some downstream targets of these fibers mediate the modulation is not known.

Neural correlates of in vivo classical conditioning

Lechner et al. (2000b) developed an in vitro preparation to study the cellular correlates of classical conditioning. The preparation consisted of the cerebral and buccal ganglia taken from animals soon after they had been classically conditioned. Brief electrical

Figure 2. Classical conditioning of feeding behavior in vivo. (A) Animals were restrained in clear plastic tubes, which facilitated stimulation of the mouth and lips. Tactile stimulation of the lips with a paintbrush served as the conditioned stimulus (CS) and food as the unconditioned stimulus (US). (B) Schematic representation of the classical conditioning stimulus protocol. A delayed pairing procedure was used in which the CS onset preceded the US onset by 3 sec. CS and US overlapped for 5 sec, after which the CS was terminated. The US was presented until the animal ingested it, but no longer than 60 sec. Unpaired training consisted of CS and US presentations at an inter-stimulus-interval (ISI) of 120 sec. The inter-trial-interval (ITI) was 4 min in all experiments. Note, the broken lines indicate that the timeline is not drawn to scale. (C) Immediately before training animals received four CS presentations (pre-test). The number of bites during this stimulation period was counted. Subsequently, animals received 10 trials of paired or unpaired training. After training the number of bites elicited by four CSs (post-test) was determined, and the change in the number of bites was calculated by subtracting the pre-test score from the post-test score. The post-test CSs were presented either 1 or 24 h after training (see Fig. 3). (Modified with permission from The Society for Neuroscience © 2000, Lechner et al. 2000a.)

Figure 3. Short- and long-term memory following classical conditioning of feeding behavior. (A) One-hour retention for classical conditioning of feeding behavior. Conditioning was measured as a change in the number of CS-elicited bites (i.e., post-test minus pre-test). Paired training produced a significantly greater change in the number of CS-elicited bites as compared to unpaired training. (B) In a separate group of animals, the retention for classical conditioning of feeding behavior was measured 24 h after training. At 24 h following training, paired training produced a significantly greater change in the number of CS-elicited bites as compared to unpaired training. (Modified with permission from The Society for Neuroscience © 2000, Lechner et al. 2000a.)

stimulation of nerve AT₄, the most medial and ventral branch of the anterior tentacle nerve (AT) which innervates the lips and contains axons of mechanoreceptors, was used as the *in vitro* representation of the CS. BMPs represented fictive feeding responses. In preparations taken from animals that previously received paired training, stimulation of the AT₄ nerve evoked a significantly greater number of BMPs as compared to preparations from animals that previously had received unpaired training. This effect was specific for AT₄-evoked BMPs, as the number

of spontaneously occurring BMPs was not different between the paired and unpaired groups. These results suggest that correlates of classical conditioning can be observed and studied in preparations of isolated ganglia. Furthermore, these data indicate that neural correlates of classical conditioning are specific to CS-evoked activity without affecting the spontaneous activity of the CPG.

Additional analysis of CS-evoked BMPs revealed that the increase in BMPs following paired training was almost exclusively attributable to an increase in the number of ingestion-like BMPs. These data indicate that classical conditioning of feeding induces specific changes in the neural circuitry that controls feeding behavior resulting in increased ingestion-like motor activity. Importantly, this effect closely mimics the effect of behavioral conditioning (i.e., an increase in the number of bites in response to tactile stimulation of the lip), thereby helping to validate the use of the *in vitro* preparation to study neural correlates of classical conditioning.

Given that classical conditioning changes activity in the neural circuitry mediating feeding behavior, several sites were investigated within the buccal CPG where plasticity might occur. Because B31/32 plays a key role in initiating BMPs (Susswein and Byrne 1988; Hurwitz et al. 1994, 1996, 2003; Dembrow et al. 2004), we investigated whether conditioning altered the intrinsic properties of B31/32 (Fig. 4A; Lechner et al. 2000b). Neither the input resistance nor resting membrane potential of B31/32 were affected by conditioning. In contrast, paired training significantly increased the excitation of B31/32 that is elicited by stimulating the CS pathway. The input from the CS pathway to B31/32 was assessed by analyzing the complex postsynaptic potential (cPSP) that is induced in B31/32 during stimulation of AT₄ (Fig. 4A1). The peak depolarization (Fig. 4A2) and integrated area (Fig. 4A3) of the AT₄-evoked cPSP in B31/32 were greater in ganglia

Figure 4. Correlates of classical conditioning in B31/32 and B51. Cerebral and buccal ganglia were removed from animals that received either paired or unpaired training. Intracellular recordings began 1–6 h following training and testing. Electrical stimulation of the AT₄ nerve (5 sec; 5 Hz) was used as an *in vitro* analog of the CS. (A) Correlates in neurons B31/32. (A1) Stimulation of AT₄ elicited a complex postsynaptic potential (cPSP) in B31/32. To quantify this cPSP, B31/32 was hyperpolarized to -80 mV and the peak depolarization and the integral (shaded region) of the cPSP were determined. To calculate the net excitatory component of the cPSP, negative integrals (i.e., hyperpolarizations; $-$ symbol) were subtracted from the depolarizing component ($+$ symbol) of the cPSP. (A2) The average peak depolarization of the cPSP was significantly larger following paired training. (A3) The average net excitation also was significantly larger following paired training. (B) Correlates in neuron B51. (B1) While B51 was current-clamped to -60 mV, the *in vitro* analog of the CS (5 sec, 5 Hz stimulation of AT₄) elicited a significantly greater number of plateau potentials in B51 in ganglia from animals that received paired training as compared to animals that received unpaired training. (B2) B51 was current-clamped to -60 mV and stimulation of AT₄ elicited a cPSP. The peak depolarization of the cPSP was measured in B51 following paired and unpaired training. (B3) The average peak depolarization was significantly larger following paired training. (B4) While B51 was current-clamped at -60 mV, the threshold for eliciting a plateau potential was determined by injecting brief (5 sec), depolarizing current pulses of increasing amplitude. The threshold for eliciting a plateau potential in B51 was determined following paired and unpaired training. (B5) The threshold for eliciting a plateau potential in B51 was increased significantly following paired training. (Modified with permission from The Society for Neuroscience © 2000, Lechner et al. 2000b, and from Nature Publishing Group © 2006, Lorenzetti et al. 2006b [http://www.nature.com/].)

taken from animals that received paired training as compared to preparations from animals that received unpaired training. These results indicate that classical conditioning of feeding behavior does not appear to affect the intrinsic properties of B31/32, but does enhance the excitation of B31/32 that is evoked by stimulation of AT₄, which in turn may underlie the increased number of CS-elicited ingestion-like BMPs in vitro and the increased number of bites in vivo.

Because B51 plays a key role in expression of ingestion-like BMPs (Nargeot et al. 1999a,b), we also investigated whether conditioning altered the activity and/or intrinsic properties of B51 (Fig. 4B; Lorenzetti et al. 2006b). In preparations taken from animals that previously received paired training, stimulation of the AT₄ nerve evoked a significantly greater number of plateau potentials in B51 as compared to preparations from animals that previously had received unpaired training (Fig. 4B1). The occurrence of plateau potentials in B51 is closely related to the expression of ingestion-like BMPs (Nargeot et al. 1999a,b). Neither the input resistance nor resting membrane potential of B51 were affected by classical conditioning. In contrast, paired training significantly increased the excitation of B51 that is elicited by stimulating the CS pathway (Fig. 4B2). The peak depolarization of the AT₄-evoked cPSP in B51 was greater in ganglia taken from animals that received paired training as compared to preparations from animals that received unpaired training (Fig. 4B3). In addition, paired training produced a significant increase in the threshold for eliciting a plateau potential in B51 (Fig. 4B4,B5). Despite this increase in the threshold for eliciting activity in B51, however, as noted above, the CS elicited more plateau potentials in B51 following paired training. These results suggest that the other factors within the neural circuit (e.g., increased CS-mediated excitation) must overcome the diminished excitability and elicit greater activity in B51, which results in a greater number of ingestion-like BMPs and, in turn, an increased number of bites in the intact animal following classical conditioning. The functional consequences of the increased threshold in B51 following paired training is unclear.

In summary, these data provide initial insights into the organization of the CS and US pathways supporting appetitive classical conditioning. Biting behavior is produced by a response system that comprises command-like neurons (e.g., CBIs) in the cerebral ganglion and CPG neurons (e.g., B31/32 and B51) in buccal ganglia. Mechanoafferents that innervate the lips mediate the CS. The US is mediated by a sensory pathway from the foregut, which, when paired with the CS, is able to modulate the CS pathway and the intrinsic properties of some elements of the circuit.

Appetitive operant conditioning

Brembs et al. (2002) developed a behavioral protocol for reward operant conditioning of feeding behavior in *Aplysia* that produces both short- and long-term memory. The in vivo protocol was developed using the consummatory phase of feeding (i.e., biting) as the operant. The first step was to design a suitable reinforcement (i.e., reward). Previous studies indicated that the anterior branch of the esophageal nerve plays an important role in learning-induced modifica-

tions of feeding in *Aplysia*. For example, the En₂ appears to mediate the US in appetitive classical conditioning (see above; Lechner et al. 2000a) and the En₂ can function as reinforcement in an in vitro analog of operant conditioning (see below; Nargeot et al. 1997). Moreover, bouts of high-frequency neural activity (~3 sec, ~30 Hz) were recorded in vivo during the ingestion of food, whereas very little activity was recorded from En₂ during biting in the absence of food (Brembs et al. 2002).

Based upon these data, an in vivo operant conditioning protocol was developed in which stimulation of the En₂ was used as reinforcement. One day prior to training, animals were implanted with a stimulating electrode on the En₂ (Fig. 5A). During training and testing, animals were allowed to move freely and an observer noted the occurrence of all bites. During contingent training, bites were immediately followed by reinforcements (brief stimulation of En₂) (Fig. 5B). During yoke training, animals received the same pattern and number of reinforcements, but these reinforcements were not correlated with the behavior of the animal (Fig. 5B). Pilot studies determined the intensity of the stimulus to be 8 VDC (10 msec pulses, 30 Hz, 3 sec). At this intensity no overt behavioral response could be observed. Conditioning was measured as the number of bites that occurred during a 5-min post-test observation period. The post-test observations occurred either immediately following training or 24 h after training. Both immediately (Fig. 6A) and 24 h (Fig. 6B) after a 10-min training session, animals in the contingent reinforcement group produced more bites than animals in the yoked-control group. Thus, brief stimulation of the En₂ can function as reinforcement during operant conditioning of feeding, and 10 min of training is sufficient to induce both short- and long-term memory.

Neural correlates of in vivo operant conditioning

The initial search for neural correlates of operant conditioning focused on B51, a cell that plays a key role in the production of ingestion-like BMPs (Nargeot et al. 1999a) and that also is a site

Figure 5. Operant conditioning of feeding behavior in vivo. (A) One day prior to training, animals were implanted with a stimulating electrode on the anterior branch of left esophageal nerve. During the training and testing phases of an experiment, the animal was allowed to move freely in a small aquarium. The aquarium contained fresh artificial seawater and a dilute extract of seawater, which helped to induce feeding behavior. An observer noted the occurrence of all bites. The observer could also deliver brief electrical shocks to the En (see panel B). (B) The black dots that are labeled Behavior represent the occurrence of a bite. The red boxes that are labeled En stim. represent the occurrence of a reinforcement (i.e., electrical stimulation of En). During contingent training, bites were immediately followed by reinforcements. During yoke training, animals received the same pattern and number of reinforcements, but these reinforcements were not correlated with the behavior of the animal. Conditioning was measured as the number of bites that occurred during a 5-min post-test observation period. The post-test observations occurred either immediately following training or 24 h after training. (Modified with permission from AAAS © 2002, Brembs et al. 2002, and from The Society for Neuroscience © 1997, Nargeot et al. 1997.)

Figure 6. Short- and long-term memory following operant conditioning of feeding behavior. (A) A significantly greater number of bites were observed immediately following training with contingent reinforcement (Cont.) as compared to the yoked training. (B) In a separate group of animals, the retention for operant conditioning was measured 24 h after training. A significantly greater number of bites were observed 24 h after training with contingent reinforcement as compared to the yoked training. (Modified with permission from AAAS © 2002, Brembs et al. 2002.)

of plasticity following classical conditioning (see above; Lorenzetti et al. 2006b). Within 1–2 h after training, the buccal ganglia were removed and the resting membrane potential, input resistance, and burst threshold of B51 were measured. B51 had a significantly lower burst threshold (Fig. 7A) and a significantly higher input resistance (Fig. 7B) in ganglia from animals that had received contingent reinforcement as compared to ganglia from animals in the yoked-control group. There was no difference in resting membrane potential between the two groups. An increase in input resistance and a decrease in threshold would increase the probability of B51 becoming active and, thus, would increase the number of ingestion-like BMPs that are expressed by the feeding circuitry. These predictions have been confirmed using an in vitro analog of operant conditioning (see below).

It is important to note that B51 is a site of plasticity following both classical and operant conditioning, but that the two training protocols produce very different changes in B51. Classical conditioning increases the threshold for eliciting a plateau potential in B51, whereas operant conditioning decreases the threshold. Moreover, operant conditioning increases the input resistance of B51, whereas classical conditioning does not affect the input resistance. These results suggest that in some respects classical and operant are mediated by fundamentally different cellular processes.

In vitro analogs of classical and operant conditioning

The development and validation of in vitro analogs is an important step in studies of the cellular and molecular mechanisms underlying associative learning. Such preparations will allow for the analyses of modifications in the intrinsic properties and/or synaptic strengths of identified elements during and following associative learning.

In vitro analog of classical conditioning

To further characterize the cellular processes involved in classical conditioning of feeding behavior, an in vitro analog of classical conditioning was developed (Mozzachiodi et al. 2003). The preparation consisted of cerebral and buccal ganglia, which were isolated from naive animals. The isolated nervous system was trained using extracellular stimulation of peripheral nerves. Stimulation of AT₄ and EN₂ were used to activate the CS and US pathways, respectively. Generally, paired and unpaired protocols were used. The number of training trials, the ITI, and the ISI of

the in vitro training protocol were selected to be similar to those used by Lechner et al. (2000a) during in vivo behavioral training.

The effects of training were assessed by subtracting the number of BMPs elicited by the pre-test CSs from the number elicited by the post-test CSs. Ganglia that received paired training had a significantly larger increase in the number of CS-evoked BMPs than the unpaired-control groups (Mozzachiodi et al. 2003; Lorenzetti et al. 2006b). There was no significant change in the frequency of spontaneous BMPs in either group. These results indicate that the paired CS/US presentations in the in vitro analog specifically increased the ability of the CS to evoke BMPs and that the in vitro training strengthened the CS-mediated pathway selectively, without affecting the spontaneous activity of the CPG.

Additional analysis of the types of BMPs evoked by CS (i.e., stimulation of AT₄) revealed that the increase was almost exclusively attributable to an increase in the number of ingestion-like BMPs (Mozzachiodi et al. 2003). Importantly, this increase in the number of CS-evoked ingestion-like BMPs is similar to the neural correlate produced by classical conditioning in vivo (Lechner et al. 2000b) and closely resembles the effect of behavioral training on the number of bites (Lechner et al. 2000a). Together, these

Figure 7. Correlates of operant conditioning in B51. Buccal ganglia were removed from animals that were trained with either contingent reinforcement or from animals that received the yoked training. Intracellular recordings began ~1.5 h following training and testing. B51 was current-clamped to -60 mV for the duration of the experiment. (A) Burst threshold. (A1,A2) Intracellular recordings from B51 cells from a matched pair of contingently reinforced and yoked-control animals. Depolarizing current pulses were injected into each B51 until the cell generated a plateau potential. In this example, a 6-nA current pulse was sufficient to generate a plateau potential in B51 from a contingently reinforced animal (A1), whereas 14 nA were required to generate a plateau potential in B51 from the corresponding yoked-control animal (A2). (A3) Summary data. B51 cells from the contingent reinforcement group required significantly less current to elicit the plateau potential. (B) Input resistance. (B1,B2) Intracellular recordings from B51 cells from both contingently reinforced and yoked-control animals. Hyperpolarizing current pulses were injected into B51 and the input resistance of the cell was measured. In this example, the membrane potential of B51 from a contingently trained animal (B1) deflected more in response to the current pulse than the potential of B51 from a yoked-control animal (B2). (B3) Summary data. B51 input resistance was significantly increased in contingently reinforced animals. (Modified with permission from AAAS © 2002, Brembs et al. 2002.)

results indicate that paired stimulation of peripheral nerves *in vitro* induces pairing-specific changes in the neural circuitry that mediates feeding behavior and that these changes persist for at least 1 h following training. Moreover, the changes produced *in vitro* are analogous to the effects of *in vivo* training.

Neural changes induced by the in vitro analog of classical conditioning

Additional investigations examined changes in the CS-mediated synaptic input to neurons B31/32 following *in vitro* training protocols. The peak amplitude and area of the CS-evoked cPSP in B31/32 were monitored before (pre-test) and 60 min after (post-test) training. The amplitude and area of the cPSP before training did not differ between the paired and unpaired groups. After paired training, however, the observed increase in the peak amplitude and the area of CS-evoked cPSP in B31/32 were significantly greater than similar measurements in preparations that received unpaired training. In contrast, the intrinsic properties of B31/32 (i.e., resting membrane potential and input resistance) did not change in either group. Paired training also produced changes in B51. Following paired training the number of CS-evoked plateau potentials in B51 was significantly increased, the peak depolarization of the CS-evoked cPSP in B51 was significantly increased, and the threshold for eliciting a plateau potential was significantly increased, but the resting membrane potential and input resistance were unaffected (Lorenzetti et al. 2006b). Once again, it is important to note that these results are similar to those following *in vivo* conditioning (see above), which suggests that both *in vitro* and *in vivo* conditioning produce similar changes in the neural circuitry that mediates feeding.

The *in vitro* analog is being used to extend the analysis of classical conditioning, in part, by investigating additional sites of plasticity (Mozzachiodi et al. 2003). Two sites were examined that failed to exhibit changes following conditioning: neurons B4/5 and CBI-2 (Mozzachiodi et al. 2003; see also Lechner et al. 2000b). One additional site of plasticity was found, however. Paired training enhances the CS-evoked input to the command-like neuron CBI-2 (Fig. 1), which leads to increased CS-evoked spike activity in CBI-2. This pairing-specific increase in the spike activity in CBI-2 may, in turn, contribute to the increased excitation in B31/32 following paired training (see Fig. 4A).

Analyzing the reinforcement pathway

Lesions of the En block classical conditioning, which suggests that the En mediates the effects of the US during appetitive classical conditioning (Lechner et al. 2000a). Moreover, histofluorescence analysis indicates that processes within the En contain dopamine (DA) (Kabotyanski et al. 1998), which suggests that DA may be a transmitter underlying the associative plasticity following classical conditioning. A number of studies in both vertebrates and invertebrates suggest that DA may play a critical role in the reinforcement pathway during various forms of associative learning (for reviews, see Berke and Hyman 2000; Kelley and Berridge 2002; Schultz 2002; Jay 2003; Harley 2004; Wise 2004). To analyze the role of DA in appetitive classical conditioning, we investigated the effects of a DA receptor antagonist, methylergonovine (e.g., Teyke et al. 1993; Nargeot et al. 1999c), on the acquisition of pairing-specific changes in the *in vitro* analog of classical conditioning (Reyes et al. 2005). Four groups of preparations were compared: Two received paired training, one with (1 nM) and the other without methylergonovine, and two received unpaired training, one with and the other without methylergonovine. The change in the number of ingestion-like BMPs during a 10-min test period 1 h after training was calculated (post-test minus pre-test). Confirming previous results (Mozzachiodi et al. 2003), in control saline, the number of BMPs was significantly larger in the preparations that received paired training group as

compared to the unpaired training protocol. In contrast, in the presence of methylergonovine, paired training had no effect on the number of BMPs. Thus, the behavioral, histofluorescence, and pharmacological data are consistent with the hypothesis that DA within the En mediates the actions of the US during classical conditioning.

In summary, these data indicate that a reduced preparation of the cerebral and buccal ganglia can be classically conditioned *in vitro* by pairing stimulation of peripheral nerves (i.e., AT₄ and En₂), which are used to mimic the CS and US, respectively. In naive preparations, weak stimulation of AT₄ (i.e., the CS) has a low probability of eliciting patterned activity in the CPG, whereas stimulation of En₂ (i.e., the US) is able to drive BMPs. The memory for classical conditioning appears to be distributed among select elements of the feeding neural network. Some elements remain unaffected (e.g., B4/5, CBI-2), whereas other elements are sites of plasticity. For example, paired training results in an enhancement of the CS-induced excitation of CBI-2, B31/32, and B51 due to modification of synaptic connections and/or intrinsic properties of neurons along the CS pathway. Thus, the CS becomes more effective in eliciting plateau potentials in B51, despite an increase in the threshold of B51, and thereby elicits more ingestion-like BMPs, which may underlie the observed increase number of bites following *in vivo* classical conditioning. Finally, DA appears to be a transmitter that mediates the US.

In vitro analog of operant conditioning

An *in vitro* analog of operant conditioning was developed (Nargeot et al. 1997; Brembs et al. 2004) and it was used to extend the investigations into the cellular mechanisms of operant conditioning. Generally, the *in vitro* analog preparation consisted of buccal ganglia isolated from naive animals (however, see Brembs et al. 2004). Sustained rhythmic activity (i.e., BMPs) was elicited by tonic, low-frequency stimulation of a peripheral buccal nerve (n2,3). During a 10-min training period, each ingestion-like BMP was reinforced by brief stimulation of En₂ (i.e., contingent reinforcement group). Generally, the control consisted of a yoked-control group, which received noncontingent presentations of En₂ stimulation. Immediately following contingent reinforcement, preparations that received contingent reinforcement produced significantly more BMPs than the yoked-control group (Nargeot et al. 1997; Brembs et al. 2004). This increase persisted for at least 24 h after the 10-min training period (Nargeot et al. 1999c; Mozzachiodi et al. 2006). Importantly, the increase in expression of BMPs was accounted for by an increase in the number of ingestion-like BMPs that were produced, i.e., the type of BMPs that was contingently reinforced.

Neural changes induced by the in vitro analog of operant conditioning

B51 is selectively associated with ingestion-like BMPs (Nargeot et al. 1999a,b) and B51 is a site of plasticity following *in vivo* operant conditioning (Brembs et al. 2002). Nargeot et al. (1999a,b) investigated whether the activity and/or properties of B51 (i.e., burst threshold and input resistance) were modified by contingent reinforcement. In preparations that received contingent reinforcement, the number of plateau potentials in B51 was increased significantly and the burst threshold in B51 was decreased significantly as compared to yoke controls. In addition, the input resistance of B51 was increased significantly following contingent reinforcement as compared to yoke controls. These changes could be induced by an explicit association of reinforcement with depolarization of B51. Importantly, these modifications were similar to those induced by the *in vivo* behavioral conditioning (see above). Taken together, these results indicate

that contingent reinforcement of ingestion-like BMPs modified the intrinsic properties of B51, which, in turn, may contribute to the increased expression of ingestion-like BMPs.

Analyzing the reinforcement pathway

During operant conditioning *in vivo*, electrical stimulation of the En served as the reinforcement and the En is rich in DA-containing processes (see above), which suggest that DA mediates reinforcement during operant conditioning. To analyze the role of DA in operant conditioning, the *in vitro* analog of operant conditioning was used to investigate the effects methylergonovine had on the changes induced by contingent reinforcement (Nargeot et al. 1999c). Four groups of preparations were compared: two contingent reinforcement groups, one with (1 nM) and the other without methylergonovine, and two yoked-control groups, one with and the other without methylergonovine. The number of ingestion-like BMPs during a 10-min test period 1 h after training was counted. Confirming previous results (Nargeot et al. 1997), in control saline, the number of ingestion-like BMPs was significantly larger in the contingent reinforcement group as compared to the yoked-control group. In contrast, in the presence of methylergonovine, contingent reinforcement had no effect on the number of ingestion-like BMPs. These results are consistent with the hypothesis that DA is a transmitter that mediates both the US during classical conditioning (see above) and the contingent reinforcement during operant conditioning.

Single-cell analog of operant conditioning

The studies described above indicate that changes in the intrinsic properties of B51 are correlated with contingent reinforcement. The cellular mechanisms that underlie this associative plasticity may be intrinsic to B51 or may be due to extrinsic factors. For example, the associative plasticity may occur in some other cell, which in turn exerts a tonic influence on B51. To address this issue, a single-cell analog of the operant conditioning was developed (Brembs et al. 2002). In this analog, individual B51 neurons were isolated from naive animals and maintained in culture. Because DA appears to play a critical role in the reinforcement pathway (see above; Nargeot et al. 1999c), reinforcement was mimicked by a brief iontophoretic puff of DA onto the neuron. Since B51 exhibits a plateau potential during ingestion-like BMPs, DA reinforcement was made contingent upon a plateau potential in the cell that was elicited by a brief depolarizing current pulse.

Two groups of cells were examined: a group that received contingent reinforcement and a control group that received non-contingent reinforcement. In the contingent reinforcement group, the cells received seven depolarizations over a 10-min training period. Each of these depolarizations elicited a plateau potential in B51, which was immediately followed by a brief (6 sec) iontophoretic puff of DA. In the control group, cells also received seven depolarizations and DA puffs, but the DA puff occurred 40 sec after the plateau potential. Contingent reinforcement produced a significant decrease in the burst threshold and a significant increase in the input resistance as compared to cells in the control group (Fig. 8). The changes produced in B51 by the single-cell analog of operant conditioning are similar to those produced by both the *in vitro* analog (Nargeot et al. 1999b) and *in vivo* training (Brembs et al. 2002). These data indicate that mechanisms intrinsic to B51 are responsible for both induction and maintenance of the biophysical changes associated with reward operant conditioning. In addition, the results suggest that aspects of operant conditioning and DA-mediated reward can be reconstituted and analyzed in single-cultured neurons.

Studies with the single-cell analog are providing some initial insights to the biochemical processes that may mediate the induction of operant conditioning (Lorenzetti et al. 2006a). The

Figure 8. Changes in B51 produced by single-cell analog of operant conditioning. (A) Burst threshold. (A1,A2) Intracellular recordings from a pair of contingently reinforced and unpaired neurons. Depolarizing current pulses were injected into B51 before (pre-test) and after (post-test) training. In this example, contingent reinforcement led to a decrease in burst threshold from 0.8 to 0.5 nA (A1), whereas it remained at 0.7 nA in the corresponding unpaired cell (A2). (A3) Summary data. The burst threshold was decreased significantly in cells that received contingent reinforcement training. (B) Input resistance. (B1,B2) Intracellular recordings from a pair of contingently reinforced and unpaired control neurons. Hyperpolarizing current pulses were injected into B51 before (pre-test) and after (post-test) training. In this example, contingent reinforcement led to an increased deflection of the B51 membrane potential in response to the current pulse (B1), whereas the deflection remained constant in the corresponding unpaired cell (B2). (B3) Summary data. The input resistance was increased significantly in the cell that received contingent reinforcement training. (Modified with permission from AAAS © 2002, Brembs et al. 2002.)

cAMP/PKA pathway appears to be involved. D1-like receptors, which are likely to be coupled to adenylyl cyclase, were identified on the membrane of B51. Moreover, inhibiting PKA with bath application of Rp-cAMP blocked conditioning. Conversely, injecting cAMP into B51 mimicked conditioning. Blocking PKC with bath application of bisindolylmaleimide also blocked conditioning. Thus, both PKA and PKC are necessary for conditioning. Because activating the PKA pathway alone can fully mimic conditioning, it appears that PKC is acting upstream of PKA and suggests that the point of convergence may be adenylyl cyclase.

These analyses of B51 also suggest that intrinsic cell-wide plasticity may contribute to appetitive operant conditioning. Moreover, the results suggest a possible general mechanism for operant conditioning. When activity in a neuron is followed by a reinforcing modulatory transmitter, biochemical and biophysical changes are induced in that neuron, which lead to an increased probability of subsequent activity in that neuron and, once activated, a more prolonged response. It is intriguing to note that similar results were observed in another *in vitro* analog of operant conditioning that utilized hippocampal slices (Stein and Belluzzi 1989; Stein et al. 1993) where contingent puffing of DA enhanced bursting activity in CA1 pyramidal cells. Although B51 is a key element of the neural circuit underlying feeding behavior in *Aplysia*, given the number of neurons in the buccal CPG, it is likely that B51 is not the only site of plasticity during

operant conditioning (nor is cell-wide plasticity likely to be the only mechanism). Thus, it will be important to expand the investigation of sites and mechanisms of plasticity that may underlie operant conditioning.

Conclusions

Feeding behaviors of a variety of gastropod mollusks can be modified by associative learning paradigms (e.g., Gelperin 1975; Croll and Chase 1977; Davis 1982; Davis et al. 1983; Susswein and Schwartz 1983; Alexander et al. 1984; Gelperin and Culligan 1984; Maximova and Balaban 1984; Mpitsos and Cohan 1986a,b; Susswein et al. 1986; Hopfield and Gelperin 1989; Kemenes and Benjamin 1989; Sahley et al. 1990; Kemenes and Benjamin 1994; Nikitin 1994; Nikitin et al. 1994; Colwill et al. 1997; Kojima et al. 1997; Benjamin et al. 2000; Jones et al. 2001; Kojima et al. 2001; Elliott and Susswein 2002; Lorenzetti and Byrne 2002; Brems 2003a,b). Learning in these gastropods has many features similar to learning in vertebrates. It can occur rapidly, and the memories following learning can persist for long periods of time. In many of these preparations, the properties of identified neurons and networks that participate in the feeding behavior are amenable to cellular analysis and the relevant features of the neuronal activity and learning are readily expressed *in vitro* (e.g., Kemenes et al. 2006; Straub et al. 2006). Thus, studies of learning in gastropods offer an opportunity to determine the cellular mechanisms and to gain key insights into processes that may also contribute to learning in vertebrates, including man.

The feeding system of *Aplysia* is providing new insights into the neural networks and subcellular mechanisms that mediate two important forms of associative learning (i.e., classical and operant conditioning), as well as providing information regarding the mechanistic inter-relationships between them. Moreover, mechanistic information on conditioning of feeding will provide insights into strategies used by the nervous system to encode and store associations. Only a few examples of associative conditioning have been analyzed mechanistically so far (for reviews, see Carew and Sahley 1986; Byrne 1987; Abrams and Kandel 1988; Crow 1988; Sahley 1995; Benjamin et al. 2000; Burrell and Sahley 2001; Christian and Thompson 2003; Crow 2004; Robleto et al. 2004; Fanselow and Poulos 2005; Maren 2005; Kim and Jung 2006). Other examples would help identify the general principles of organization among them.

Some interesting similarities and differences between appetitive classical and operant conditioning have been identified (Table 1). One similarity is the nature of the reinforcement pathway and its transmitter. The En is the reinforcement pathway for operant conditioning and the US pathway for classical conditioning. This pathway appears to use DA as a transmitter. Also, the direct application of DA can mimic the reinforcement. These results are consistent with the view that DA can mediate the US/reinforcement for appetitive forms of both classical and op-

Figure 9. Schematic diagram of the different contingencies during operant (A) vs. classical (B) conditioning. The occurrence of a plateau potential in cell B51 is believed to be critical for the production of ingestion-like BMPs (Nargeot et al. 1999a,b) and presumably for feeding in freely behaving animals. Thus, the occurrence of an ingestion-like BMP and/or a plateau potential in B51 were the neuronal equivalents of an operant and were immediately followed by stimulation of the anterior branch of the esophageal nerve (En₂). As a result of this contingent reinforcement, the input resistance and excitability of B51 were increased (upward arrows). Presumably, the changes in B51 were induced by an interaction between some trace of the activity in B51 (e.g., calcium influx during the plateau potential) and some DA-induced process (e.g., activation of adenylyl cyclase). During classical conditioning, the contingency was between stimulation of AT₄ (the CS) and En₂ (the US). As a result of this pairing, the excitability of B51 decreased (downward arrow). Presumably, the changes in B51 were induced by an interaction between a process induced by a yet-to-be-identified transmitter released during the CS and a DA-induced process. Despite the opposite effects that classical and operant conditioning had on the excitability of B51, both protocols increased the number of plateau potentials, the number of ingestion-like BMPs, and the number of bites following conditioning. Thus, some other modifications (e.g., increased synaptic input) must override the decreased excitability of B51 following classical conditioning.

erant conditioning. B51 is a cellular locus for the changes induced by both operant and classical conditioning. Both *in vivo* and *in vitro* operant conditioning increase the input resistance and the excitability of B51. These two changes make B51 more likely to be active, which can account for the increased expression of ingestive behavior following contingent reinforcement. In contrast, no pairing-specific changes in the input resistance of B51 were observed following either *in vivo* or *in vitro* classical conditioning. Also, both operant and classical conditioning modified the threshold level for eliciting plateau potentials in neuron B51, but in opposite directions, revealing a fundamental difference in the cellular mechanisms underlying these two forms of associative learning.

B51 appears to be a coincidence detector for both the CS-US association (classical conditioning) and the contingency between ingestive behavior and reinforcement (operant conditioning) (Fig. 9). Because DA likely mediates both the US and the reinforcement, a key problem is to elucidate the mechanisms that lead to the induction of opposite effects on the burst threshold. One possibility is that the coincidence detector for classical conditioning involves an association between a transmitter released by the CS and DA (Fig. 9B), whereas, for operant conditioning, the coincidence detector involves an association between the cellular effects of plateau potentials in B51 and DA (Fig. 9A). Exploring these pathways and their interactions will provide important insights into the molecular logic of operant conditioning and classical conditioning.

The finding that DA is involved in both appetitive classical conditioning and reward operant conditioning supports current theories that DA signals encode the value and probability of reward (for reviews, see Schultz 2002; Jay 2003; Chinta and Andersen 2005; Harley 2004; Kelley 2004; Hyman 2005; Tobler et

Table 1. Comparison of classical and operant conditioning

	Classical conditioning	Operant conditioning
Number of bites	Increase	Increase
Number of ingestion-like BMPs	Increase	Increase
Reinforcement pathway	En	En
Reinforcement transmitter	Dopamine	Dopamine
Number of plateau potentials in B51	Increase	Increase
Resting membrane potential of B51	No change	No change
Input resistance of B51	No change	Increase
Burst threshold of B51	Increase	Decrease

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al. 2005; Wise 2005). Dopamine plays a critical role in associative learning in mammals (Schultz 1997; Liu et al. 2004; Wise 2004). Pharmacological blockade of DA receptors interferes with the acquisition of conditioned behaviors produced by classical and operant conditioning procedures (Schultz 1997; Eyny and Horvitz 2003; Andrzejewski et al. 2005), and reinforcing stimuli activate DAergic neurons (Shizgal and Murray 1989; Schultz 2001). Moreover, the activity of DAergic neurons is correlated with changes in the parameters of reinforcing stimuli such as probability, uncertainty, and magnitude (Cromwell and Schultz 2003; Fiorillo et al. 2003; Martin-Soelch et al. 2003; Montague et al. 2004; Stefani and Moghaddam 2006) and stimulation of DAergic systems can serve as reward (Wise 2005). Dopamine appears to play a similarly important role in associative learning in *Aplysia*, and feeding behavior of *Aplysia* provides a useful model system for future studies of cellular and molecular mechanisms of DA-mediated reinforcement.

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