Nonassociative and Associative Modification of Head-Waving Produced by Aversive Tentacular Stimuli in *Aplysia*

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Abstract

Head-waving, a spontaneously occurring exploratory and appetitive behavior of the marine mollusc *Aplysia*, provides an opportunity to examine mechanisms of learning expressed in a nonreflexive behavior. The present study explores nonassociative and associative forms of learned modification of head-waving produced using an aversive stimulus as reinforcement. Experiments on intact, freely behaving animals demonstrate that training with electric shock as an aversive unconditioned stimulus, delivered unilaterally to the anterior tentacles, produces a learned shift in head-waving behavior away from the side on which shock was applied. This behavioral change is a novel learned behavioral response that is influenced by the topographic location of an aversive stimulus. Furthermore, training with application of tentacle shock reinforcement, contingent upon the animal's head position, produces operant conditioning of head-waving. Thus, anterior tentacle shock is effective as an aversive reinforcer for both nonassociative and operant learning expressed in the head-waving behavior of *Aplysia*.

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Introduction

Animal model systems, including invertebrates such as the marine mollusc *Aplysia californica*, have been of great value in investigating the cellular and molecular mechanisms of learning and memory. For the most part, behavioral research aimed at a cellular analysis of learning in *Aplysia* has concentrated on the modification of defensive withdrawal reflexes. For example, through the use of a combined behavioral and cellular approach, it has been possible to achieve a detailed understanding of the mechanisms of sensitization and classical conditioning of gill and siphon withdrawal (for review, see Castellucci and Schacher 1990; Byrne et al. 1991; Marcus et al. 1994; Byrne and Kandel 1996). However, reflexes constitute only a subset of the complete behavioral repertoire of *Aplysia*. More complex behavioral patterns, such as those involved in feeding, locomotion, and exploration, are important as well but have been studied less extensively from the perspective of the cellular mechanisms of learning. One such behavior in *Aplysia* is head-waving, a spontaneously occurring behavioral pattern consisting of cyclical sweeps of the head and neck from side to side with a period of ~60 sec. This behavior is a component of the feeding and egg-laying motor programs and also occurs as a spontaneous exploratory behavior (Kupfermann 1970, 1974; Kupfermann and Carew 1974; Bablanian et al. 1987). Head-waving can be readily studied in the laboratory, and the motor pathways and peripheral musculature involved in producing the behavior have, at least in part, been identified (Cook and Carew 1989a; Kuenzi and Carew 1994b); thus, this behavior presents an opportunity to examine learning in a more complex, nonreflexive behavior while retaining the analyti-

cal advantages of a simple invertebrate model system.

Head-waving consists of head movements in the horizontal and vertical planes, as well as lengthening and shortening of the neck (Kuenzi and Carew, 1994a). Our experiments focused on only one component of this behavior, head movement in the horizontal plane, for two reasons. First, horizontal movement is the most consistently rhythmic component of the overall behavior. Head position alternates from side to side about once per minute, whereas other types of movements, such as those in the vertical plane, occur with lesser frequency and regularity. Thus, within a 10-min observation period, several cycles of lateral movement are exhibited, providing an approximately symmetric baseline against which the effects of training can be assessed. Second, it is the horizontal component of the behavior that has been shown previously to be modifiable by experience (Cook and Carew 1986, 1989a; Kuenzi and Carew 1991).

Prior work has demonstrated that head-waving may be modulated by a specific form of associative learning, operant conditioning (Cook and Carew 1986, 1989a). However, to date, modification of head-waving by experience such as the noncontingent presentation of an aversive unconditioned stimulus (US) has not been examined. Such a form of learning may be of ethological relevance to the organism in navigating and exploring its environment and might also serve as a useful model for examining mechanisms of learning expressed in nonreflexive behaviors. Another issue that has not been explored extensively is the way in which the topographic location of a US may be important in producing specific learned changes in behavior. In the analysis of learning in *Aplysia*, it has generally been thought that the effects of an aversive US are rather widespread and that the location of the stimulus carries minimal relevance in determining the behavioral outcome. There are, however, several instances in which the location of an aversive US has been shown to be important for learning. First, certain training procedures (such as strong unilateral shock to the animal's body) can produce sensitization of the siphon and tail-withdrawal reflexes that is side-specific, or even site-specific (Scholz and Byrne 1987; Walters 1987). Second, the topography of siphon withdrawal responses can be directionally modified, depending on the location of the aversive US used in a nonassociative sensitization paradigm (Erickson and Walters 1988). These examples show that there is prece-

dent for directional modification of reflex behaviors by noncontingent, aversive stimulation in *Aplysia*. However, the occurrence of this type of plasticity in head-waving has not been examined previously. Thus, the first aim of the present experiments was to examine the ability of a focal, aversive US (unilateral tentacle shock) to produce nonassociative learning, expressed as a directional change in head-waving behavior.

The second objective was to explore an associative form of learning, operant conditioning, produced by a tentacle shock US. Prior work has shown that head-waving can be conditioned in an operant manner, using bright light as aversive reinforcement (Cook and Carew 1986, 1989a). To further extend the analysis of operant learning in *Aplysia*, we wished to determine whether anterior tentacle shock can serve as an effective form of aversive reinforcement in an operant conditioning paradigm. The primary motivation for this experiment was to identify a type of reinforcement that would be amenable to mechanistic, cellular analysis. In addition, the demonstration that operant conditioning of head-waving is not restricted to a specific modality of reinforcement strengthens the applicability of this paradigm as a general model for operant learning.

Materials and Methods

ANIMALS

Wild-collected adult *A. californica* (150–300 grams) were obtained from commercial suppliers on the Pacific coast (Marinus, Pacific Biomarine, or Alacrity). Animals were maintained in aquaria (1000 liters) filled with aerated artificial seawater (Instant Ocean, Aquarium Systems, Mentor OH) at 15°C and were fed seaweed (Nagai Sushinori, Japan, ~0.75 gram daily).

OBSERVATION OF HEAD-WAVING AND TRAINING PROCEDURES

Aplysia were tested and trained individually in a square transparent plastic tank (35 cm/wall), filled to a depth of ~23 cm with Instant Ocean (Fig. 1A). Animals were suspended in the tank by nylon tethers attached to stainless-steel hooks inserted

Figure 1: Experimental observation of head-waving. (A) Schematic drawing of *Aplysia* suspended in the observation tank and engaged in head-waving. The superimposed outlines of the animal's body are tracings taken from a videotaped experiment at 3-sec intervals. (B) Delivery of shock reinforcement during training. Shock was applied with a hand-held probe, either to the lateral tip of one side of the anterior tentacles (B1) or centrally (B2).

through the dorsal margin of each parapodium. Care was exercised to support the animal's body and avoid pinching or stretching the parapodia during this procedure. Once suspended, animals reliably engaged in head-waving behavior for a period of at least 30 min. Because the *Aplysia* body is close to neutrally buoyant, animals float in a horizontal position with very little strain placed on the suspending hooks, and minimal active behavior is required to maintain a neutral, horizontal position. Head-waving usually consists of bilateral cycles of motion, but animals sometimes exhibited successive flexures toward the same side from the center position. Some head-waving movements involve full-body flexure while others consist of independent movements of the head and neck. Animals spent ~75% of the time engaged in lateral head movements on either side of the center position. With careful handling, animals usually did not exhibit any signs of distress in this procedure. Animals that produced defensive behaviors such as inking or escape locomotion in response to han-

dling (~20% of all animals) were removed from the testing apparatus and rerun at least 1 day later.

Each experiment consisted of three successive 10-min phases: Baseline, Training, and Test, which were separated by intervals of ~1 min. An observer monitored head-waving continuously during the Baseline and Test phases, scoring head position as "left" or "right" when there was noticeable lateral flexure of the neck, and "middle" at all other times. The same observer scored all animals and was blind to experimental conditions. Head position scores were indicated by a three-position switch and sampled at 1-sec intervals. During Training, shock reinforcement, either lateral (Fig. 1B1) or central (Fig. 1B2) was delivered by another experimenter to the tentacles via bipolar electrodes (0.5 mm diam., 6 mm long, separated by 2 mm), mounted on a hand-held probe. The standard shock stimulus was 26 V, 60 Hz AC, for 100 msec, which was sufficient to produce a brief withdrawal of the tentacles and partial withdrawal of the head but did not elicit signs of a generalized defensive response, such as inking or whole-body withdrawal. In experiments using noncontingent reinforcement, one side was selected randomly to be the shocked side, and lateral shock was delivered on either a 30-sec fixed-interval schedule, or a randomized schedule with a mean 30-sec interstimulus interval.

In operant conditioning experiments, shock was applied to the center of the anterior tentacles in a contingent manner during the Training phase (Fig. 1B2). One side was randomly selected to be the punished side, and shock was delivered whenever the animals' head crossed to that side (after a 5-sec delay, to allow the experimenter time to bring the shock probe into position). Repeated shocks were delivered at 5-sec intervals if the animal's head remained on the "punished" side. The timing of reinforcement was under the control of a computer used to record head position data. Following training, head-waving was monitored in the absence of reinforcement by the original observer during the 10-min Test period. It is common in operant conditioning experiments to employ yoked control subjects to control for nonassociative effects of training. In the experiment reported here, the change in behavior is evaluated with regard to the training that the animal received (i.e., in reference to the "punished" and "unpunished" sides). It is not possible to quantify the behavior of control animals using the same measure, as a yoked control animal would not have a "punished" or

“unpunished” side for behavioral assessment during testing. However, the design of the experiment controls against nonassociative effects: The only way that animals can systematically shift their head-waving away from the side associated with punishment is by detecting the contingency of reinforcement upon head position. For these reasons, yoked controls were not included in this experiment.

STATISTICAL ANALYSIS

Group data are expressed as means $\pm$ standard errors of the mean. Changes in the lateral component of head-waving were assessed by comparing the total amount of time spent flexing toward each side in the Baseline and Test periods. A composite score was calculated for each animal to express the net shift in head-waving away from the shocked side (or punished side, in the conditioning experiment). The score reflects the increase in time head-waving on the nonshocked side (Test minus Baseline) plus the decrease in time on the shocked side (Baseline minus Test). This composite score was

chosen because it has been employed in previous studies of head-waving behavior and is sensitive to changes in head-waving produced by learning (Cook and Carew 1986, 1989a). A T-test for related means was used to determine whether these scores differed from zero, indicating a significant change in the lateral component of head-waving behavior. The standard level of significance was at least $P < 0.05$. All probability values are two-tailed.

Results

MODIFICATION OF HEAD-WAVING BY NONCONTINGENT LATERAL TENTACLE SHOCK

Ten animals were used in this experiment. During baseline testing, before any stimuli were delivered, animals spent roughly equal amounts of time head-waving toward both sides (Fig. 2A; shocked side, 274 ± 26 sec; nonshocked side, 239 ± 23 sec). At the beginning of each experiment, one side was selected randomly to be the site where shock was received. Training consisted

Figure 2: Modification of head-waving by tentacle shock (random interval). (A) Histograms indicate the time spent head-waving toward the shocked and nonshocked sides during 10-min periods before (Baseline, open bars) and after (Test, shaded bars) training. The composite learning score (hatched bar) is the decrease in head-waving toward the shocked side plus the increase on the nonshocked side. The composite score differed significantly from zero [$t(9) = 2.32$, $P < 0.05$], indicating that animals biased their head-waving away from the shocked side. (B,C) Two measures of the overall level of head-waving activity, total head-waving (the sum of the time spent head-waving toward both sides; B) and an activity index (equal to the number of changes in head position; C), indicate that there is no substantial decrease in head-waving behavior as a result of training.

of lateral anterior tentacle shock delivered on a randomized schedule. The average interval between shocks was ~30 sec, with a minimum interval of 5 sec. Animals received a mean of 19 ± 1.1 shocks during the training period. An important aspect of this training procedure is that it was non-associative: Shock was delivered irrespective of head position. Thus, there was no opportunity for operant conditioning, as shock was not contingent upon any aspect of the animal's behavior. Following training, in the Test phase, animals exhibited a shift in head-waving away from the shocked side: The time spent head-waving toward the shocked side decreased substantially while time on the non-shocked side increased slightly, producing a significant net change in head-waving behavior [Fig. 2A; composite score = 119 ± 51.3 sec, $t(9) = 2.32$, $P < 0.05$]. Thus, animals trained with lateral shock subsequently shift their head-waving away from the side of the body on which shock was applied. This change in behavior represents a novel form of learning expressed in the head-waving behavior of *Aplysia* that is under the control of the topographic location of an aversive stimulus.

An important issue in interpreting these findings is the exact nature of the behavioral change produced by training. There are several ways in which animals might alter their behavior to produce a shift in head position away from the shocked side. For example, animals might continue head-waving with the same frequency but reduce the "dwell time" of head-waving on the shocked side. Or animals could reduce the number of movements toward the shocked side, or exhibit a combination of these modifications. Alternatively, the same pattern of results could be produced by a static postural shift in head position. In the limit, animals might turn away from the shocked side reflexively and cease head-waving completely; this type of response would result in a large positive learning score.

To exclude the last possibility, two measures were used to determine whether there was a change in the overall level of head-waving activity following training in these experiments. First, total head-waving was calculated as the sum of time spent head-waving toward both sides. Total head-waving decreased slightly in the Test period, but this change was not statistically significant (Fig. 2B). Second, an activity index was computed for each animal, which was equal to the total number of changes in head position in each phase of the

experiment (i.e., the number of times that the animal's head position differed from its position 1 sec earlier). This measure would indicate whether animals stopped head-waving and "locked up" in a fixed position, or otherwise reduced the frequency of their head movements. Figure 2C shows the levels of this activity index for the Baseline and Test phases. There was virtually no change in activity in the Test phase. Thus, animals do not cease or substantially diminish head-waving activity following training with anterior tentacle shock. These data, in combination with qualitative behavioral observations, support the interpretation that animals continue to head-wave with a similar level of activity following training and that the side-specific change in behavior consists of a dynamic shift in the allocation of time spent head-waving toward the shocked and non-shocked sides.

To specify the nature of the behavioral alteration more precisely, several additional analyses were conducted. First, the number of times in the Baseline and Test phases that each animal moved its head to the shocked side was determined. For the purposes of this analysis, if the animal's head was already on the shocked side when scoring began, this event was counted as a crossing to the shocked side. Second, the average dwell time of head movements to the shocked side was calculated as the time on the shocked side in each phase of the experiment, divided by the number of crossings to the shocked side in that phase. These measures indicate that the net change in head-waving produced by training with anterior tentacle shock reflects the combination of both a reduction in the number of crossings to the shocked side (Baseline, 12.1 ± 1.6 ; Test, 10.3 ± 2.1), and a decrease in the average dwell time on the shocked side. (Baseline, 25.4 ± 3.1 sec; Test, 18.7 ± 5.0 sec).

To extend and generalize these results, another experiment was conducted that was essentially a replication, except that shock was delivered on a fixed schedule, at 30-sec intervals, to one side of the tentacles (randomly selected). Twenty animals were trained with lateral shock in this manner. As in the previous experiment, training produced a decrease in head-waving toward the shocked side and an increase in head waving toward the non-shocked side (Fig. 3A), giving rise to a statistically significant net change in behavior [composite score = 173 ± 82.2 sec, $t(19) = 2.10$, $P < 0.05$]. The net change in head-waving follow-

A

B

C

Figure 3: Modification of head-waving by tentacle shock (fixed interval). (A) Histograms indicate the time spent head-waving toward the shocked and nonshocked sides during 10-min periods before (Baseline, open bars) and after (Test, shaded bars) training. The composite learning score (hatched bar) is significantly different from zero, indicating that training with lateral shock on a fixed-interval schedule produces a shift in head-waving away from the side to which shock was delivered. (B,C) As in the previous experiment, overall levels of head-waving show only a slight decrease following training.

ing training was attributable to both a reduction in the number of crossings to the shocked side (Baseline, 15.2 ± 2.7 ; Test, 5.2 ± 0.83) and a decrease in the average dwell time on the shocked side (Baseline, 48.3 ± 29.3 sec; Test, 24.0 ± 7.8 sec). Again, there was no significant change in overall levels of head-waving behavior, as measured by total head-waving and number of changes in head position (Fig. 3B,C). Thus, under conditions of either random or fixed-interval reinforcement, noncontingent tentacle shock delivered laterally produces a side-specific, learned change in head-waving behavior.

OPERANT CONDITIONING WITH TENTACLE SHOCK REINFORCEMENT

In the experiments described above, non-associative learning was induced by lateralized tentacle shock. In the next experiment we wished to determine whether a similar paradigm, but one in which shock was contingent on the animals' head-waving behavior, might be capable of inducing a form of associative learning, operant condi-

tioning. In this experiment, animals (total number 14, in two independent replications) received contingent reinforcement with shock to the center of the tentacles when they flexed toward the side that had been selected randomly to be punished. An example of the head-waving data and reinforcement schedule for a single animal is shown in Figure 4A. Conditioned animals received an average of 21 ± 1.4 shocks during the 10-min Training session. During Training, animals gradually reduced head-waving toward the negative side and received progressively fewer reinforcements as Training proceeded (Fig. 4B). Following Training, animals maintained a significant bias in head-waving during the 10-min test period, as compared to Baseline (Fig. 4C). Specifically, animals reduced head-waving toward the punished side and increased head-waving toward the "safe" side [composite score = 170 ± 51.5 sec, $t(13)=3.31$, $P < 0.01$]. Because the side to be punished was selected randomly, and the reinforcement itself contained no directional information, this side-specific change in head-waving indicates that animals were sensitive to the contingency of reinforcement.

Figure 4: Operant conditioning of head-waving using tentacle shock reinforcement. (A) Example of an individual subject's head-waving behavior and reinforcement schedule during the training period. The animal's head position [(R) right; (M) middle; (L) left] is shown by the continuous trace. In this case, head-waving to the right was punished. Reinforcement, indicated by tick marks, was delivered when the animal's head crossed to the right side, and at 5-sec intervals if the head remained on the right side. (B) Acquisition of operant conditioning. The mean number of reinforcements delivered during the training phase of the experiment is shown. As training proceeded, animals ($n = 14$) reduced head-waving toward the negative side and received progressively fewer reinforcements. (C) Retention of the conditioned response after training. Histograms indicate the mean time spent head-waving toward the punished and nonpunished sides during 10-min periods before and after training. (Open bars) Baseline; (shaded bars) Test. The composite learning score, which is equal to the decrease in head-waving toward the punished side plus the increase on the nonpunished side, indicates a significant shift in head-waving behavior away from the side associated with punishment.

Discussion

NONCONTINGENT MODIFICATION OF HEAD-WAVING: A NOVEL LEARNED BEHAVIORAL RESPONSE IN *APLYSIA*

The results of the first two experiments demonstrate a novel learned behavioral response in *Aplysia*, modification of head-waving by noncontingent aversive reinforcement. This type of learning is characterized by a directional shift in head-waving away from the stimulated side; thus, the behavioral change resembles a form of learned avoidance of an aversive stimulus. Noncontingent modification of head-waving is an apparently nonassociative form of learning, as it is produced by a training procedure that does not involve explicit pairing or contingency of stimuli. However, although this paradigm specifically was intended to

examine nonassociative learning, opportunities exist for associative learning as well, and associative mechanisms may contribute to this form of learning. For example, the experimental apparatus in which training and testing were conducted is a novel context for the animals, which could act as a conditioned stimulus. Prior work has shown that *Aplysia* exhibit context-US conditioning: Animals that receive shock in a novel context show enhanced defensive reflexes when tested in that context (Colwill et al. 1988). Thus, a possibility in the present experiments is that an association is formed between the context and the tentacle-shock US. Therefore, the shift in head-waving may be, at least in part, a conditioned response to the context. If this is the case, it would be expected that animals would show less of a shift in head-waving if they were tested in a context different from the one in which training occurred. It will be

an interesting question for future research to determine the extent to which contextual effects contribute to this form of learning.

RELATION OF NONCONTINGENT MODIFICATION OF HEAD-WAVING TO OTHER FORMS OF LEARNING IN *APLYSIA*

Several points of comparison may be made between noncontingent modification of head-waving and two other types of learned, directional modification of behavior in *Aplysia*: side-specific sensitization of defensive withdrawal reflexes and pseudoconditioning of directional siphon responses. Side-specific sensitization refers to enhancement of withdrawal responses elicited by stimuli on one side of the body, which is produced by training with aversive stimulation on that side (Scholz and Byrne 1987). Noncontingent modification of head-waving and side-specific sensitization are produced by essentially similar training procedures; in both cases, training consists of noncontingent application of an aversive US to one side of the body. However, noncontingent modification of head-waving and side-specific sensitization differ in the nature of the behavioral alteration produced by training. First, side-specific sensitization is expressed in a discrete withdrawal reflex, in response to subsequent stimulation of the trained side of the animals, rather than an ongoing, patterned behavioral output. Second, side-specific sensitization involves facilitation of response amplitude, rather than a change in the directional form of the response. However, because the study of nonassociative learning in *Aplysia* has tended to focus on changes in reflex amplitude, the possibility cannot be excluded that directional changes do occur in side-specific sensitization but have thus far escaped detection.

In terms of the response alteration produced by learning, noncontingent modification of head-waving bears a greater resemblance to siphon pseudoconditioning, which is a change in the form of siphon responses produced by noncontingent training with shock (Erickson and Walters 1988). Both types of learning involve a directional change in response topography that is under the influence of the site of application of the training US. In both cases, the change in response resembles the alteration that would be seen as a result of conditioning, yet the production of this change does not require associative training. Both noncontingent

modification of head-waving and siphon pseudoconditioning may be regarded as forms of learning that encode the location or direction of a noxious stimulus. However, the types of responses involved in these two forms of learning imply quite different behavioral strategies for responding to a threatening stimulus. Although learned changes in head-waving presumably serve to avoid contact with a noxious stimulus, the apparent function of directional siphon responses is to direct ink, which is expelled in response to a strong noxious stimulus, toward the source of the stimulus, such as a predator (Erickson and Walters 1988).

A final distinction between noncontingent modification of head-waving and other directional forms of learning, such as sensitization or pseudoconditioning, concerns the intensity of stimulation that is required to produce learning. The training US used in the present experiments is an electric shock that is apparently fairly mild, as it does not elicit dramatic defensive behaviors such as inking. In contrast, the stimuli (usually tail shock) used in sensitization training are typically delivered at levels that induce inking and escape locomotion and sometimes protracted whole-body withdrawal. Thus, noncontingent modification of head-waving appears to have a lower threshold for induction than other forms of learning that are produced by aversive stimuli in *Aplysia*.

OPERANT CONDITIONING OF HEAD-WAVING WITH TENTACLE SHOCK REINFORCEMENT

The present results demonstrate that electric shock to the anterior tentacles is an effective reinforcer for operant conditioning of head-waving. This finding holds considerable promise for advancing the analysis of the mechanisms of operant learning in *Aplysia*. Prior work has shown that head-waving may be conditioned in an operant manner using bright light as reinforcement (Cook and Carew 1986, 1989a), and peripheral pathways necessary for photic reinforcement have been identified (Cook and Carew 1989b; Cook et al. 1991). However, the central neurons that mediate the reinforcing effects of light have not been identified. Tentacle shock activates known neural elements, including identified cerebral ganglion sensory neurons (Rosen et al. 1979); thus, it will be possible to conduct further cellular analyses of the reinforcement pathways mediating operant conditioning. As described by Fitzgerald and Carew (this

issue), several forms of plasticity have been identified in the cerebral ganglion sensory neurons, and it will be interesting to explore the possibility that such forms of plasticity may contribute to operant conditioning.

Operant conditioning of head-waving and modification of head-waving by noncontingent tentacle shock share the same input pathway and produce similar behavioral changes. This raises the interesting possibility that similar types of neural plasticity or modification may underlie the acquisition and behavioral expression of learning in both cases. For example, plasticity in afferent pathways that mediate tentacle shock reinforcement may contribute to both forms of learning. Furthermore, both types of learning could involve similar changes in the neural circuitry that produces head-waving. A predominant theme in the analysis of learning and memory in *Aplysia* has been that the understanding of nonassociative forms of learning and plasticity has proven important in elucidating the mechanisms of associative learning. For example, activity-dependent synaptic facilitation, a mechanism that has been proposed to contribute to classical conditioning of withdrawal reflexes in *Aplysia*, is an amplification of heterosynaptic facilitation, that underlies sensitization of the same reflexes (Hawkins et al. 1983; Walters and Byrne 1983). These findings suggest that nonassociative and associative forms of learning in this system are mediated by common "building blocks" of plasticity at the cellular level. Therefore, there is considerable promise that the mechanistic investigation of noncontingent modification of head-waving may provide insights into instrumental learning as well.

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References

Bablanian, G.M., K.R. Weiss, and I. Kupfermann. 1987. Motor control of the appetitive phase of feeding behavior in *Aplysia*. *Behav. Neural Biol.* **48**: 394–407.

Byrne, J.H. and E.R. Kandel. 1996. Presynaptic facilitation

revisited: state and time dependence. *J. Neurosci.* **16**: 425–435.

Byrne, J.H., D.A. Baxter, D.V. Buonomano, L.J. Cleary, A. Eskin, J.R. Goldsmith, E. McClendon, F.A. Nazif, F. Noel, and K.P. Scholz. 1991. Neural and molecular bases of nonassociative and associative learning in *Aplysia*. *Ann. N.Y. Acad. Sci.* **627**: 124–149.

Castellucci, V.F. and S. Schacher. 1990. Synaptic plasticity and behavioral modifications in the marine mollusk *Aplysia*. *Prog. Brain Res.* **86**: 105–115.

Colwill, R.M., R.A. Absher, and M.L. Roberts. 1988. Context-US learning in *Aplysia californica*. *J. Neurosci.* **8**: 4434–4439.

Cook, D.G. and T.J. Carew. 1986. Operant conditioning of head-waving in *Aplysia*. *Proc. Natl. Acad. Sci.* **83**: 1120–1124.

———. 1989a. Operant conditioning of head-waving in *Aplysia*. I. Identified muscles involved in the operant response. *J. Neurosci.* **9**: 3097–3106.

———. 1989b. Operant conditioning of head-waving in *Aplysia*. III. Cellular analysis of possible reinforcement pathways. *J. Neurosci.* **9**: 3115–3122.

Cook, D.G., M. Stopfer, and T.J. Carew. 1991. Identification of a reinforcement pathway necessary for operant conditioning of head waving in *Aplysia californica*. *Behav. Neural Biol.* **55**: 313–337.

Erickson, M.T. and E.T. Walters. 1988. Differential expression of pseudoconditioning and sensitization by siphon responses in *Aplysia*: Novel response selection after training. *J. Neurosci.* **8**: 3000–3010.

Fitzgerald, K. and T.J. Carew. 1997. Multiple forms of facilitation produced by aversive tentacular stimuli in cerebral ganglion sensory neurons of *Aplysia*. *Learn. & Mem.* (this issue).

Hawkins, R.D., T.W. Abrams, T.J. Carew, and E.R. Kandel. 1983. A cellular mechanism of classical conditioning in *Aplysia*: Activity-dependent amplification of presynaptic facilitation. *Science* **219**: 400–404.

Kuenzi, F.M. and T.J. Carew. 1991. Identification of neuronal pathways mediating phototactic modification of head-waving in *Aplysia californica*. *Behav. Neural Biol.* **55**: 338–345.

———. 1994a. Head waving in *Aplysia californica*: I. Behavioural characterization of searching movements. *J. Exp. Biol.* **195**: 35–51.

———. 1994b. Head waving in *Aplysia californica*: II. Functional anatomy and muscular activity during behaviour. *J. Exp. Biol.* **195**: 53–74.

Kupfermann, I. 1970. Stimulation of egg-laying by extracts of

neuroendocrine cells (bag cells) of abdominal ganglion of *Aplysia*. *J. Neurophysiol.* **33**: 877–881.

———. 1974. Feeding behavior in *Aplysia*: A simple system for the study of motivation. *Behav. Biol.* **10**: 1–26.

Kupfermann, I. and T.J. Carew. 1974. Behavioral patterns of *Aplysia californica* in its natural environment. *Behav. Biol.* **12**: 317–337.

Marcus, E.A., N.J. Emptage, R. Marois, and T.J. Carew. 1994. A comparison of the mechanistic relationships between development and learning in *Aplysia*. *Prog. Brain Res.* **100**: 179–88.

Rosen, S.C., K.R. Weiss, and I. Kupfermann. 1979. Response properties and synaptic connections of mechanosensory neurons in cerebral ganglion of *Aplysia*. *J. Neurophysiol.* **42**: 954–974.

Scholz, K.P. and J.H. Byrne. 1987. Long-term sensitization in *Aplysia*: Biophysical correlates in tail sensory neurons. *Science* **235**: 685–687.

Walters, E.T. 1987. Site-specific sensitization of defensive reflexes in *Aplysia*: A simple model of long-term hyperalgesia. *J. Neurosci.* **7**: 400–407.

Walters, E.T. and J.H. Byrne. 1983. Associative conditioning of single sensory neurons suggests a cellular mechanism for learning. *Science* **219**: 405–408.

Received October 11, 1996; accepted in revised form February 3, 1997.

Nonassociative and associative modification of head-waving produced by aversive tentacular stimuli in *Aplysia*.

K K Fitzgerald, C A Takacs and T J Carew

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References

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