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Higher-order Associative Processing in *Hermissenda* Suggests Multiple Sites of Neuronal Modulation

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Abstract

Two important features of modern accounts of associative learning are (1) the capacity for contextual stimuli to serve as a signal for an unconditioned stimulus (US) and (2) the capacity for a previously conditioned (excitatory) stimulus to “block” learning about a redundant stimulus when both stimuli serve as a signal for the same US. Here, we examined the process of blocking, thought by some to reflect a cognitive aspect of classical conditioning, and its underlying mechanisms in the marine mollusc *Hermissenda*. In two behavioral experiments, a context defined by chemosensory stimuli was made excitatory by presenting unsignalled USs (rotation) in that context. The excitatory context subsequently blocked overt learning about a discrete conditioned stimulus (CS; light) paired with the US in that context. In a third experiment, the excitability of the B photoreceptors in the *Hermissenda* eye, which typically increases following light–rotation pairings, was examined in behaviorally blocked animals, as well as in animals that had acquired a normal CS–US association or animals that had been exposed to the CS and US unpaired. Both the behaviorally blocked and the “normal” learning groups exhibited increases in neuronal excitability relative to unpaired animals. However, light-induced multiunit activity in pedal nerves was suppressed following normal conditioning

but not in blocked or unpaired control animals, suggesting that the expression of blocking is mediated by neuronal modifications not directly reflected in B-cell excitability, possibly within an extensive network of central light-responsive interneurons.

Introduction

Many modern theories of associative learning reflect the belief that a conditioned stimulus (CS) must provide unique information about the occurrence of an unconditioned stimulus (US) if that CS is to acquire associative strength (Rescorla 1988; cf. Matzel et al. 1988; Papini and Bitterman 1990). This provision stems from seminal observations that a stimulus (CS₁) that has been paired previously with a US can “block” subsequent associations to a second stimulus (CS₂) when the two stimuli are reinforced in compound with the same US (Kamin 1969). This failure to condition to CS₂ is said to arise because of the redundant predictive information provided by that stimulus. Like a discrete CS, background contextual cues that have been paired previously with a US may block acquisition of associative strength by a discrete stimulus. According to the formulation of Rescorla and Wagner (1972), contextual stimuli are postulated to compete with discrete CSs for the limited associative strength supported by a particular US. Therefore, each US occurrence that is uncorrelated with the CS (or which precedes CS training) increases the associative strength of the context and may block subsequent learning about a CS trained in that context. Other theoretical accounts of contextual blocking have also focused on the capacity for a previously trained CS to disrupt

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learning about a novel CS when the two are trained in compound. For example, Pearce and Hall (1980) and Sutherland and Mackintosh (1971) propose that blocking may result from a disruption of CS processing during the compound conditioning phase of a blocking experiment. As a general class, these models suggest that CSs (contexts or discrete cues) *compete* for associative strength. In contrast to these formulations, Gibbon and Balsam (1981) assert that a US can support a finite amount of expectancy that is spread uniformly across a training session. However, contextual stimuli and discrete CSs each acquire associative strength based on their contiguous relationship with the US (also see Miller and Schachtman 1985). At the time of testing, the *response strength* of the individual stimuli in a blocking experiment is determined by a *comparison* of the relative expectancies evoked by each stimulus. The relative merit of these contrasting views of blocking has served as a point of conceptual debate for nearly two decades, and various experiments have provided support for each of these theoretical treatments (for review, see Durlach 1989).

Modulation of associative learning by discrete or contextual stimuli has been observed in several invertebrate model systems, including demonstrations of blocking in *Limax* (Sahley et al. 1981) and a decrement in conditioning resulting from the addition of unsignalled USs during a conditioning session in *Hermisenda* (Farley 1987). Typically, the explanation for these phenomena has been based on nonassociative interference (e.g., habituation) as opposed to associative interactions between stimuli as described above. Nevertheless, although such demonstrations suggest that invertebrates might have the capacity to learn about contextual stimuli, direct demonstrations of such learning have been infrequent. However, recent examples of context learning by *Aplysia* have been reported. Using various behavioral measures, Colwill et al. (1988a) demonstrated the ability of *Aplysia californica* to associate a context with an aversive US (shock). Additionally, Colwill et al. (1988b) reported that contextual cues can serve as a discriminative stimulus for the expression of an association between a CS (tactile stimulation) and US (shock).

Recently, our laboratory has demonstrated the ability of *Hermisenda* to form an association between a diffuse chemosensory context and a US presented within that context (Rogers et al. 1996). In three experiments, *Hermisenda* were

exposed to brief unsignalled presentations of high-speed rotation (US) in one of two contexts that were defined by diffuse chemosensory stimuli (shrimp or scallop extract). Animals were then tested by exposing them to small pieces of the shellfish meat and recording the latency to strike at the meat. In all experiments, the latency to strike at the cue used to define the context paired with rotation was significantly reduced relative to the latency to strike at the cue used to define the neutral context. However, in a two-choice test where the animal could approach the conditioned or neutral stimulus, the animals regularly avoided the stimulus paired with rotation. Moreover, if following conditioning the animals were presented with an unsignalled rotation in the conditioned context or the neutral context, the animals exhibited more effective clinging to the substrate in the conditioned context, suggesting that it "prepared" the animal for the aversive US. In total, these findings indicate that *Hermisenda* are capable of making differential associations between diffuse contextual stimuli and biologically relevant events that occur within their boundaries.

In the present experiments we examined the capacity of a context (defined by a chemosensory stimulus) made excitatory by unsignalled US (rotation) presentations to subsequently block learning about a discrete CS (light) paired with the US in that context. Having observed blocking, we then examined specific cellular correlates of the CS-US association at the level of sensory receptors (B photoreceptors) and motor output (pedal nerve 1) to localize the site of blocking within the *Hermisenda* nervous system. These data suggest that previous descriptions of network interactions thought to underlie learning in *Hermisenda* are insufficient to account for these more complex associative processes, and further, the data address some of the differential predictions made by the various accounts of blocking described above.

Experiment 1

Blocking refers to the finding that less responding develops to CS₂ when it is conditioned in compound with a good predictor of the US (i.e., CS₁), relative to when CS₁ is associatively neutral. The effect can be obtained using either discrete or contextual stimuli as the blocking agent. In fact, the disruption of learning observed following US preexposure (Randich and Lolordo 1979) in ver-

tebrates is largely attributable to contextual conditioning (Balsam 1985; Randich and Ross 1985), a feature of associative learning that may be preserved in invertebrate preparations (e.g., Abramson and Bitterman 1986; Colwill et al. 1988a). *Hermisenda* are also capable of forming an association between a nominally “unsignalled” US and the context in which that US is presented (Rogers et al. 1996). In experiment 1 we investigated the ability of a contextual association formed by unsignalled US presentations to modulate subsequent responding to a discrete CS paired with a US in the presence of that excitatory context. This was accomplished using diffuse chemosensory cues to define the contextual setting and unsignalled rotation as the US, as described previously by Rogers et al. (1996).

Materials and Methods

SUBJECTS

Seventy-two adult *Hermisenda crassicornis* were obtained from Sea Life Supply Co. (Sand City, CA) and were housed individually in perforated centrifuge tubes (50 ml). Animals were maintained on a 10-hr/14-hr light–dark cycle in a recirculating tank of Instant Ocean (IO; Aquarium Systems, Mentor, OH; 12°C). During the light phase, a 25-W light was filtered through yellow acetate such that a uniform intensity of $20 \mu\text{W} \times \text{cm}^{-2}$ was recorded at the surface of the water. In this and subsequent experiments, behavioral testing began after at least a 3-day acclimation period in the laboratory but no later than 1 week following the arrival of the animals. All behavioral training and testing were conducted during the middle 8 hr of the light phase of the diurnal maintenance cycle. Throughout behavioral training and testing, animals were fed a portion (~1.5 mg) of Hikari Gold Fish food just prior to the dark cycle on alternating days.

APPARATUS

The conditioning apparatus consisted of six circular chambers milled into a single piece of clear Plexiglas mounted atop a white Plexiglas base. Each chamber was partially filled with 25–30 ml of 12°C IO taken from the animal’s home tank (or IO + extract), with one animal confined to each chamber. A clear Plexiglas cover was fastened over the chambers, thereby isolating each

chamber. These chambers were then mounted atop an orbital mixer (model 4600, Lab-Line Instruments, Inc., Melrose Park, IL) that, when operated at ~300 rpm, produced a 4-mm orbital displacement and served as the US. Positioned 34.5 cm directly above the chambers was a 40-W light source, which illuminated the chambers with a uniform intensity of $600 \mu\text{W} \times \text{cm}^{-2}$ and served as the CS during conditioning. To evaluate foot contraction to light (CS), animals were placed into an apparatus consisting of six alleys ($15.3 \times 1.0 \times 0.6$ cm; L×W×D) that were milled into a single piece of clear Plexiglas (18.5×21.5 cm; L×W). These tracks were then mounted 15 cm above a CCD video camera (model AVC-D7, Sony Corp.) for observation and recording during testing. All apparatus were housed in a light- and sound-proof incubator maintained at 13°C.

BEHAVIORAL CONDITIONING AND TESTING

Stock pastes of the chemosensory cues were prepared on the first day of training by blending 50 ml of IO with ~110 mg of scallop or shrimp. The stock was frozen after use and thawed each day to make fresh solutions. For the final conditioning solutions, the stock paste was diluted 3:100 (vol/vol) in IO and then filtered to removed large food particles.

In three replications, the contextual blocking effect was investigated behaviorally. Four groups (see Table 1) received 3 days of experience with a context defined by the presence of a specific chemosensory cue (shell-fish extract). For three of these groups, (B+)A:P, (A+)A:P, and (A+)A:UP, the chemosensory context was reinforced each day by 50 US presentations (rotation, designated by “+”, ITI = 30 sec) but differed with respect to the chemosensory cue used to define the context, that is, shrimp (B) extract versus scallop (A) extract. In contrast, group (A-)A:P was preexposed to the scallop chemosensory context for an equal duration (25 min/day) but without US presentations (nonreinforced, designated by “-”). Following preexposure training, all groups received either paired (“P”) or unpaired (“UP”) CS–US presentations within the scallop extract context (A). Following 5 min of dark adaptation, paired groups were exposed to 60 presentations of light and rotation consisting of a 4-sec light (CS) coterminating with a 3-sec rotation (US; ISI = 1 sec). The unpaired group received identical stimuli except that

Table 1: Experiment 1 training protocol

Group	US pre-exposure	CS/US training	Test
(B+)A:P	B + US	A : paired	CS alone
(A+)A:P	A + US	A : paired	CS alone
(A+)A:UP	A + US	A : unpaired	CS alone
(A-)A:P	A alone	A : paired	CS alone

(A) Scallop context; (B) shrimp context.

the presentations were explicitly unpaired, with the unpaired USs occurring midway between CS presentations. All groups were trained in this phase using a 90-sec ITI. In the test phase, configuration changes of the animal's foot in response to the light (CS) alone was assessed in straight alleys 23–24 hr following the final training trial. Animals were confined to a single IO-filled track (six in all) by fixing an opaque Plexiglas cover over the apparatus. Following 5 min of dark adaptation, all animals were exposed to an 8-sec presentation of the light (CS). Each animal was then given 5 min of further dark adaptation, followed by a second CS presentation. The length of the foot was measured from video tape at the onset of the light and just before the offset, with the change in foot length over the 8-sec period serving as our measure of foot contraction (see Matzel et al. 1989). When a measure was obtained for both presentations, a mean of the two was calculated and served as the single datum for those animals.

STATISTICAL ANALYSIS

Data from experiment 1 were subjected to a one-way analysis of variance (ANOVA) comparing the percent change in foot length during CS presentation across treatment conditions. Planned comparisons of individual means were conducted based on the overall mean-square error term of the ANOVA.

Results and Discussion

Owing to the nature of the testing apparatus (see above), data could not be obtained for 19 of the 72 animals conditioned. This was the result of animals either turning or at the end of a track at the time of a test light presentation that obscures changes in foot length elicited by light.

Figure 1 presents the mean percent change in foot length over the 8-sec test interval for each group. Consistent with their unpaired training, group (A+)A:UP ($n=15$) failed to exhibit any foot contraction to the light CS. Instead, a slight foot extension was noted that is characteristic of the unconditioned response to light (i.e., phototaxis). In contrast, group (B+)A:P ($n=12$), for which the preexposure USs and CS–US pairings occurred in different contexts, exhibited a conditioned foot contraction in response to the light CS. The foot contraction exhibited by this group indi-

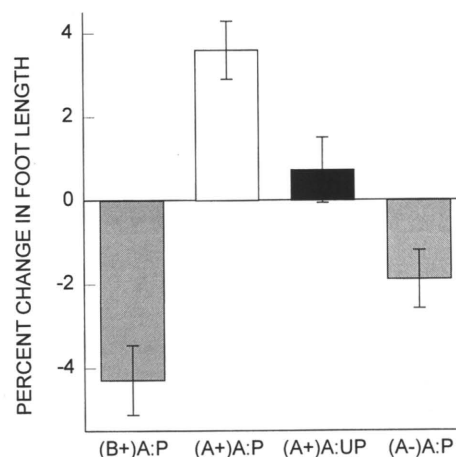


Figure 1: Conditioned foot contraction following contextual blocking. The mean percent change in foot length during an 8-sec test light (CS). Animals were preexposed to the US (rotation) in one of two chemosensory-defined contexts (A = scallop extract; B = shrimp extract). Plus and minus signs indicate whether the context was reinforced (+) or nonreinforced (–) during US preexposure. Subsequently, all groups received CS (light)–US training within context A in either a paired (P) or unpaired (UP) fashion. Contextual blocking was observed in group (A+)A:P in that they fail to exhibit any light-induced foot contraction [see groups (B+)A:P and (A-)A:P], despite paired CS–US conditioning in phase 2 of training. Brackets indicate s. e.

cates that despite preexposure to the US, the context shift between the preexposure phase and the CS-US training phase was sufficient to allow development of a typical (see Lederhendler et al. 1986) conditioned response, thus demonstrating context specificity (relative to group (A+)A:P) and the associative nature [relative to group (A+)A:UP] of the affects of US preexposure. A conditioned foot contraction in response to light was also exhibited by group (A-)A:P ($n=15$), which was preexposed to the CS-US training context with no US presentations. Finally, group (A+)A:P ($n=11$), for which the preexposure and CS-US training context were equivalent, failed to develop a conditioned foot contraction to light. For these animals, the context-US association that develops during the preconditioning phase (Rogers et al. 1996) was sufficient to disrupt subsequent learning and/or expression of the conditioned response. These observations were confirmed using one-way ANOVA in which a main effect of conditioning history was obtained, $F(3,49)=17.90$, $P<0.001$. Planned comparisons of individual means were conducted, and a difference was observed between groups (B+)A:P and (A+)A:P, $F(1,49)=46.97$, $P<0.001$, as well as between groups (A+)A:UP and (A-)A:P, $F(1,49)=6.74$, $P<0.05$. Further comparisons revealed a difference between groups (A-)A:P and (B+)A:P, $F(1,49)=5.10$, $P<0.05$. It is interesting to note that although a conditioned foot contraction was observed for group (A-)A:P, the magnitude of the response appears attenuated relative to group (B+)A:P. It has been observed in the vertebrate literature that the affective value of an aversive US may be enhanced when presented against a background of appetitive USs (Fowler 1971). Because in the present case the conditioning context has an appetitive value (see Rogers et al. 1996) and is novel at the time of conditioning for group (B+)A:P, this may represent a similar type of effect in *Hermisenda*.

Finally, group (A+)A:P (behaviorally blocked) exhibited a *larger* foot extension than group (A+)A:UP, $F(1,49)=7.03$, $P<0.05$. Whether this difference represents a real effect of conditioning history will be addressed in experiment 2. It would appear though that the foot extension of group (A+)A:UP is smaller than might be expected based on other reports in which the foot extension exhibited by unpaired animals was more similar to the behavior of group (A+)A:P (e.g., Lederhendler et al. 1986; Matzel et al. 1992).

In total, these results demonstrate contextual blocking in an invertebrate system using diffuse chemosensory contextual cues. Previous research has demonstrated the ability of *Hermisenda* to form context-US associations using parameters and stimuli equivalent to the preexposure phase of experiment 1 (Rogers et al. 1996). Theories of learning predict that such an association should block subsequent learning or conditioned responding to a stimuli (e.g., light) trained in that context (e.g., Rescorla and Wagner 1972; Gibbon and Balsam 1981). Of the three groups that received paired light and rotation training in conditioning phase 2, only group (A+)A:P did so in a context previously paired with the rotation US. Consistent with the theoretical predictions and previous research with vertebrate and invertebrate preparations, no evidence of a conditioned response (i.e., foot contraction) was observed in group (A+)A:P. One potential limitation of experiment 1 is the exposure of the respective groups to different chemosensory cues. Although previous work has demonstrated the ability of both shrimp (B) and scallop (A) cues to support context-US associations (Rogers et al. 1996), a better balanced design would be one in which we demonstrate contextual blocking in animals that have received exposure to both contextual cues, differing only in regard to their reinforcement history (i.e., differential contextual conditioning). These issues were addressed further in experiment 2.

Experiment 2

Experiment 1 demonstrated contextual blocking of a CS-US association by a diffuse chemosensory context that had been paired previously with the US. These findings do not address the process underlying contextual blocking. As discussed previously, both the Rescorla-Wagner model (Rescorla and Wagner 1972) and the Scalar Expectancy Theory (SET; Gibbon and Balsam 1981) agree that excitatory contextual cues may directly modulate the formation or expression of conditioned responding within that context. However, these formulations differ with regard to the mechanism by which blocking is manifest (also see Pearce and Hall 1980; Miller and Schachtman 1985). Specifically, Rescorla and Wagner (1972) dictate that the formation of a light (CS)-rotation (US) association will be disrupted when pairings occur in an excitatory context, while Gibbon and

Balsam (1981) allow for the normal formation of the CS–US association in an excitatory context. Blocking, according to SET, is manifested as a disruption in the expression of the intact association.

Previous research has identified a network interaction in *Hermisenda* through which the CS–US association could be disrupted by an excitatory context. Specifically, chemosensory pathways are known to interact synaptically with and inhibit type B photoreceptors of the eye (Alkon et al. 1978). This class of photoreceptor is known to exhibit an increase in excitability following paired conditioning that is strongly correlated with the magnitude of the conditioned response elicited by the light CS (Crow and Alkon 1980; Farley et al. 1983; Matzel et al. 1992). Briefly, the output of B photoreceptors is enhanced following conditioning and is thought to attenuate positive phototaxis through an inhibition of neighboring type A photoreceptors that mediate positive phototactic behavior (for review, see Alkon 1983; Goh and Alkon 1984). Moreover, the B photoreceptors have been presumed to directly mediate a contraction of the animal's foot, although in the untrained animal this response is inhibited by reciprocal projections from the normally dominant type A photoreceptor (Lederhendler et al. 1986). Thus, the increase in excitability (i.e., input resistance) of the B photoreceptors is thought to causally contribute (at least in part) to associative alterations in light-initiated behaviors and has served as a primary cellular index of associative learning in numerous studies (e.g., West et al. 1982; Matzel and Rogers 1993; Rogers et al. 1984).

Based on our knowledge of the neural network of *Hermisenda*, context blocking might occur if the inhibitory synaptic interaction between the chemoreceptors and B photoreceptors were enhanced by context conditioning, affectively blocking learning through a disruption of CS (light) processing. For instance, a facilitation of this intersensory inhibition by the formation of a context–US association could feasibly attenuate the capacity of light to sufficiently depolarize the B cell within the excitatory context, rendering the CS ineffective (cf., Talk and Matzel 1996). This speculation, of course, centers on the assumption that context conditioning results in a potentiation of cells within the chemosensory network, that these same cells project onto the B photoreceptors, and that the inhibition of the B photoreceptors by the chemosensory input is sufficient to attenuate the depolarizing light response. Confir-

mation of this scenario would indicate that blocking in this preparation results from a disruption of CS processing (e.g., Sutherland and Mackintosh 1971; Pearce and Hall 1980). It is also reasonable to expect that blocking may arise from a disruption of US processing as specified by Rescorla and Wagner (1972), although this possibility does not appear consistent with our present understanding of network interactions in *Hermisenda*. For instance, Alkon et al. (1978) observed a chemosensory-mediated inhibition of spontaneous inhibitory postsynaptic potentials (IPSPs) in hair cells but determined that this resulted indirectly from a chemosensory-mediated inhibition of second-order visual cells within the optic ganglion, which monosynaptically inhibit ipsilateral hair cells. Consequently, chemosensory inputs actually result in some facilitation of hair cell (US) activity by initiating a reduction of basal inhibition induced by activity in optic ganglion cells. Thus, any potentiation of chemosensory cells as a result of context conditioning is most likely to enhance the interaction between hair cells and the B photoreceptors. Parenthetically, optic ganglion cells also interact with type B photoreceptors through an excitatory projection. Inhibition of these optic ganglion cells by chemosensory inputs could then further influence CS processing through the removal of this excitatory input and a retardation of the long-lasting depolarization observed in B cells at light offset.

Experiment 2 addressed the possibility that the CS–US association was disrupted during the contextual blocking procedure by evaluating B-cell excitability following conditioning within a previously reinforced context. A disruption of the increase in B-cell excitability by the blocking procedure would be consistent with either the Rescorla–Wagner (1972) or Pearce–Hall (1980) descriptions of blocking, although such a demonstration would not distinguish between these views. For this experiment, animals were differentially conditioned to one of two contexts. Subsequently, a single session of light–rotation training ensued in either an excitatory or neutral context. Animals were then tested behaviorally and the excitability of B photoreceptors was evaluated electrophysiologically. It must be stressed (also see Discussion) that although the B photoreceptor is generally thought to contribute to the generation of the conditioned response, several distinct sites of plasticity have now been identified in *Hermisenda* (e.g., Farley et al. 1990; Frysztać and Crow 1993; Mc-

Phie et al. 1993), as well as within compartments of the B photoreceptor (Schuman and Clark 1994). Other sites are likely to be determined as our knowledge of this animal expands. Consequently, our use of the B photoreceptor as a cellular index of associative modifications is necessarily incomplete (see Discussion). Nevertheless, it will allow us to draw some preliminary conclusions regarding the cellular mechanisms by which blocking is expressed.

Materials and Methods

SUBJECTS

Thirty-six naive *Hermissenda*, housed and maintained under the same general conditions as in experiment 1, served as subjects in this experiment. All behavioral conditioning, testing, and electrophysiological recordings were conducted during the middle 8 hr of the light phase.

BEHAVIORAL CONDITIONING AND TESTING

The behavioral conditioning and testing apparatus, as well as the chemosensory cues, were the same as used in experiment 1. As with experiment 1, animals were trained and tested in three phases, although a differential conditioning procedure was used (see Table 2) to examine the generality of the blocking effect to other procedures. During phase 1 (US preexposure phase), animals were exposed to two contexts (scallop extract or IO alone) each day for 3 days. The two 25-min exposures were separated by an interval of 3 hr, and the order of exposures were balanced across days. For each group, one context was reinforced using 50 presentations of rotation (3-sec US; ITI = 30 sec). During the second exposure, the animals were placed into the second context for an equal duration but without US exposure (i.e., nonrein-

forced). Thus, groups (A+/IO-)A:P and (A+/IO-)A:UP received context-US pairings in context A (scallop), whereas group (IO+/A-)A:P received them in IO only. No dark adaptation during the preexposure phase was given in this experiment in order to reduce the duration of chemosensory exposure. All animals were returned to their respective home tubes during all delays. CS-US training in phase 2 of conditioning was conducted in context A using the same parameters described for experiment 1. Twenty-four hours following the final training trial of phase 2, the foot contraction elicited by the CS alone was examined for all groups as described previously (see Experiment 1). In addition to the behavioral measure of the CS-US association (i.e., CS-elicited foot contraction), neurophysiological characteristics of the type B photoreceptor were investigated 1.5–4.5 hr following behavioral testing. Owing to the length of time necessary to study all of the animals (i.e., 1.5–4.5 hr), care was taken to have samples from each group distributed throughout this interval. Prior to any electrophysiological analysis, the animals' group assignments were coded such that the experimenter was unaware of any particular subject's treatment history.

ELECTROPHYSIOLOGY

Just prior to electrophysiological analysis, nervous systems containing the visual-vestibular network but surgically isolated from chemoreceptors were removed and pinned to a glass slide, subjected to proteolysis (Protease Type IX, Sigma Chemical; 10 mg/ml artificial seawater) for 8–10 min at 22°C, and rinsed in 5°C artificial seawater. All preparations were perfused continually throughout in vitro study with artificial sea water (ASW; 20°C, 1–5 ml/min) prepared from (in mM) NaCl 430, CaCl₂ 10, MgCl₂ 50, and KCl 10, buff-

Table 2: Differential conditioning schedule

Group	Pre-exposure		CS/US Training	Test
	session 1	session 2		
(A+/IO-)A : P	A + US	IO alone	A : paired	CS alone
(A+/IO-)A : UP	A + US	IO alone	A : unpaired	CS alone
(IO+/A-)A : P	IO + US	A alone	A : paired	CS alone

(A) Scallop context; (IO) Instant Ocean context. Session order was alternated across 3 days of pre-exposure.

ered with Tris to a pH of 7.4–7.5. For current-clamp recordings, glass microelectrodes were pulled to a tip resistance of 20–30 M Ω in ASW using a KAc (3.0 M) fill and connected to an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). In medial B photoreceptors, input resistance and current-elicited spikes, which are indirect measures of cell excitability, were measured by passing a series of 500-msec current pulses (–0.6, –0.4, –0.2, 0.2, 0.4, and 0.6 nA) through the recording electrode using a balanced-bridge circuit. Membrane resistance was indicated by the current-induced voltage deflection measured 300–400 msec after the onset of the pulse. Voltage responses were recorded on a Brush Pen Recorder for off-line analysis.

STATISTICAL ANALYSIS

Behavioral data from experiment 2 were subjected to a one-way ANOVA comparing the percent change in foot length during CS presentation across treatment conditions. When input resistance was evaluated, the voltage response of the B photoreceptors to +0.6 nA current injection was used as the index of input resistance. Data were subjected to a one-way ANOVA in which the single factor was conditioning history of the animal. Data evaluating current-elicited spikes were compared using two-way repeated measures ANOVA, in which factor 1 was the conditioning history and factor 2 was the input current (0.2, 0.4, 0.6 nA). All other measures were compared using one-way ANOVA. Planned comparisons of individual means were conducted based on the overall mean-square error term of the ANOVA's.

Results and Discussion

In two replications, the contextual blocking effect was investigated behaviorally using differential conditioning of the chemosensory contexts. For reasons discussed in experiment 1, data were not obtained from 7 of the 36 animals tested.

Figure 2 presents the behavioral data for experiment 2 in which the mean percent change in foot length over the 8-sec test interval is plotted for each group. As in experiment 1, animals receiving unpaired light and rotation training in phase 2, (A+/IO–)A:UP ($n = 8$), failed to exhibit any foot contraction to light but instead displayed the characteristic foot extension of naive animals. Also

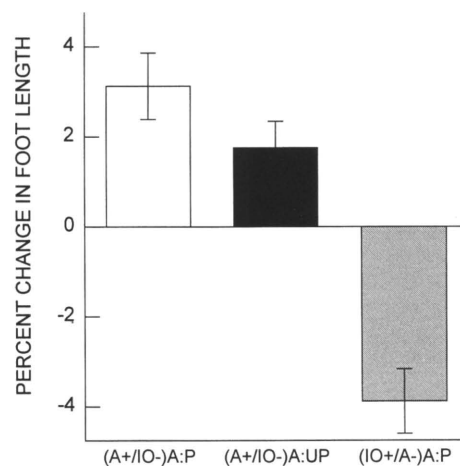


Figure 2: Conditioned foot contraction following differential behavioral conditioning. The mean percent change in foot length during an 8-sec test light. Animals were preexposed to two contexts (A = scallop extract; IO = instant ocean sea water) each day. For each group, one context was reinforced (+) by unsigned US (rotation) presentations. During the second exposure, animals were placed into the second context but without US presentations (–). Subsequently, all groups received CS (light)–US training within context A in either a paired (P) or unpaired (UP) fashion. Contextual blocking is evident in group (A+/IO–)A:P for whom the preexposure and CS–US training contexts were equivalent but not in group (IO+/A–)A:P who experienced a context shift between sessions. Brackets indicate s. e.

consistent with experiment 1, group (IO+/A–)A:P ($n = 10$), for whom the US preexposure and CS–US training contexts differed, displayed a normal development of a conditioned foot contraction. This finding replicates that of experiment 1 but with the methodological variation that these animals were exposed to both contexts (IO and scallop) during the preexposure phase of training. Therefore, the experiential history of group (IO+/A–)A:P differed from the other groups only along one dimension, that of which context was reinforced. It should be noted that for group (IO+/A–)A:P the IO served as the background stimulus during both preexposure and testing phases of the experiment. This procedure could possibly complicate our interpretations in that the CR magnitude may reflect the summed contribution of an excitatory context added to the CS-evoked behavioral response. However, our use of the percent change in foot length was meant to account in part for any possible contribution of the context to changes in foot length and provide

a more direct measure of the light-induced CR. Also consistent with experiment 1 we found that group (A+/IO-)A:P ($n=8$) failed to display any conditioned foot contraction to the light CS. Despite paired conditioning during phase 2 of training, the association formed between context A and the US during the preexposure phase was sufficient to block subsequent learning about the light CS when CS-US pairings occurred within that excitatory context. These observations were confirmed by one-way ANOVA in which a main effect of conditioning history was found, $F(2, 26) = 29.0$, $P < 0.001$. Planned comparisons of the individual means found significant differences between groups (A+/IO-)A:P and (IO+/A-)A:P, $F(1,26) = 53.52$, $P < 0.001$, and groups (A+/IO-)A:UP and (IO+/A-)A:P, $F(1,26) = 29.59$, $P < 0.001$.

Groups (A+/IO-)A:P and (A+/IO-)A:UP did not differ from one another. This later result contrasts with the significant difference observed between comparable groups in experiment 1. Despite a lack of statistical significance, it should be noted that the same basic trend in responding was obtained as in experiment 1; that is, blocked animals exhibited a greater foot extension than did unpaired animals. On the basis of the results of these two experiments, it is impossible to say with any certainty whether this tendency represents a real effect of training history or simply a sampling error. It is interesting nonetheless that the blocking we observe is so complete. This has typically not been the case in vertebrate conditioning preparations that have used either contextual or punctate stimuli to block subsequent learning (e.g., Kammin 1969; Matzel et al. 1987). It is unclear at present whether learning in *Hermisenda* is more easily disrupted by the blocking procedure or whether we have serendipitously chosen particularly affective parameters for the establishment of blocking.

To investigate conditioning-induced biophysical changes in type B photoreceptors, animals were prepared for intracellular electrophysiological analysis 1.5–4.5 hr following behavioral testing. Figure 3A presents mean membrane voltage responses in the B photoreceptor as a function of input current for all three groups. These curves illustrate the resistance of a cell's membrane to input current, which directly relates to the excitability of the cell. As is evident from the curves, groups (A+/IO-)A:P and (IO+/A-)A:P both exhibit an increase in membrane resistance at all

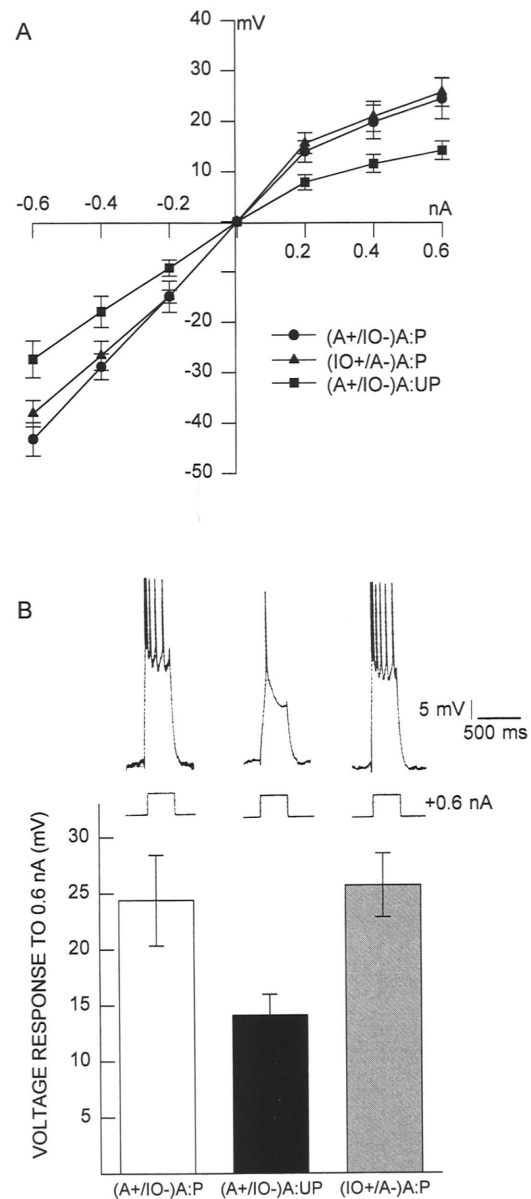


Figure 3: Changes in B photoreceptor input resistance following contextual blocking. (A) The current–voltage (I–V) relationship in type B photoreceptors. This relationship serves as an index of cellular excitability and is correlated with the formation of a CS–US association at the level of the photoreceptor. (B) The mean B-cell voltage response to 0.6 nA of input current. Representative voltage records are shown above each bar; these three responses were observed in response to +0.6-nA current injections from a holding potential of –60 mV. As was evident from A, animals experiencing paired light–rotation training [i.e., (A+/IO-)A:P and (IO+/A-)A:P] exhibited an increase in membrane resistance relative to their unpaired control, suggesting the presence of an intact CS–US association in behaviorally blocked animals. Brackets indicate s.e.

current levels relative to the unpaired control condition, that is, (A+/IO-)A:UP. To summarize these data, the mean voltage response for a single input current (+0.6 nA) and a representative voltage trace from each group is presented in Figure 3B. At this input current, groups (A+/IO-)A:P ($n=9$) and (IO+/A-)A:P ($n=8$) exhibited a 73% and 82% increase in resistance, respectively, relative to group (A+/IO-)A:UP ($n=8$). These differences were confirmed by one-way ANOVA in which a main effect of conditioning history was obtained, $F(2,22)=3.94$, $P<0.05$. Planned comparisons revealed a significant increase in resistance in both paired groups relative to the unpaired control condition, $F_s(1, 22)\geq 5.45$, $P_s<0.05$, but not relative to each other, $F(1,22)=0.01$ [not significant (N.S.)].

In addition to input resistance, the number of action potentials elicited by the 500-msec positive current pulses (from a holding potential of -60 mV) was measured and is summarized in Figure 4. As would be expected based on the increase in excitability reported above, there is a tendency for

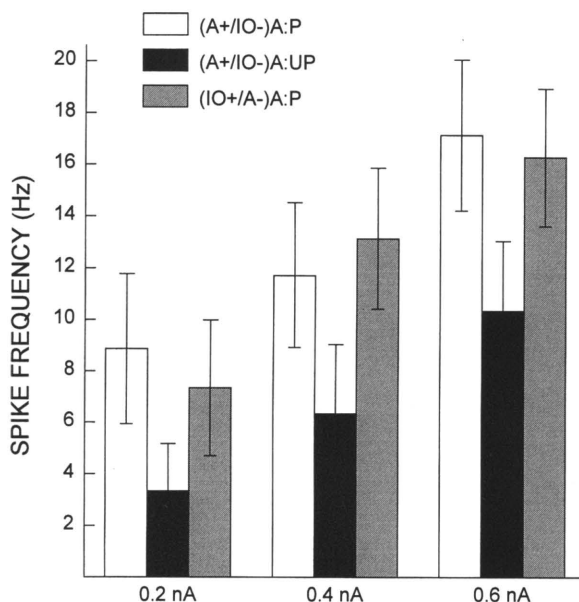


Figure 4: Current-elicited spike frequency in B photoreceptors. Following behavioral conditioning using the contextual blocking procedure, the excitability of B photoreceptors was tested using 0.2–0.6-nA current steps. The figure presents mean data for the number of current-elicited action potentials. The greater number of spikes for paired (P) groups, relative to unpaired (UP) animals, is consistent with an increase in input resistance across the cell membrane (see Fig. 3). Brackets indicate s. e.

a higher frequency of current-elicited spikes in both of the paired groups, (A+/IO-)A:P and (IO+/A-)A:P, relative to the unpaired control, (A+/IO-)A:UP. This observation was supported by a two-factor ANOVA, where treatment history and magnitude of current injection (0.2, 0.4, and 0.6 nA) served as factors. A significant difference between groups was observed, $F(2,59)=4.23$, $P<0.05$, and group history did not interact with the magnitude of injected current, $F(4,59)=0.13$ (N.S.). Thus, an increase in input resistance produces an increase in spike frequency for a constant level of injected current. This increase in current-elicited spike activity is consistent with previous reports demonstrating an increase in light-induced firing frequency following conditioning (Farley and Alkon 1982; cf. Crow 1985), although these studies employed longer duration light steps.

In addition to current-elicited spikes, we also measured spikes elicited by a 4-sec light presentation. Surprisingly, in the present experiments we found no enhancement of light-induced spiking in either group (A+/IO-)A:P or in group (IO+/A-)A:P relative to the unpaired control condition, $F(2,17)=1.62$ (N.S.). The mean spike rates (in Hz, \pm s. e.) were 20.0 (\pm 2.0), 15.7 (\pm 1.2), and 16.1 (\pm 3.01), respectively. Because spike rate represents one of the final pathways whereby a change in excitability can affect the behavior of a neural network, the lack of difference observed here may suggest a relatively minor role for the B photoreceptor in the storage of the light-rotation associative memory (cf. Farley et al. 1983; Matzel et al. 1992). However, given the difference observed in current-elicited spike rates, we are inclined to believe that the absence of light-elicited differences in spike rate represents a sampling error attributable to the small number of observations. Several other possibilities do exist however. First, it is possible that spike width (and not frequency) increases following conditioning in *Hermisenda*. This is unlikely though, given that paired presentations of light and rotation result in a reduction of the fast, transient K^+ current (I_A) and the Ca^{2+} -dependent K^+ current (I_{K-Ca} ; Alkon et al. 1985), both of which should affect spike frequency to a greater degree than spike width (for a description, see Rogawski 1985). A second possibility is that we simply cannot detect changes in spike frequency in the intact, isolated eyes. In contrast to injected current, the test light that we employ stimulates all of the photoreceptors of the eye. Because the five photoreceptors of the eye

are mutually inhibitory, it is not possible to say how changes in excitability in one cell will affect spike rates in that cell when neighboring cells are also firing. Resolution of these issues will require a more complete understanding of the neural network of *Hermissenda* as well as recordings from synaptically isolated cells.

In experiment 2, contextual blocking was demonstrated as in experiment 1 but was done so with a different methodology, that is, differential contextual conditioning. One of the primary goals of this experiment was to correlate these behavioral findings with known cellular changes in B photoreceptors related to the formation of CS-US associations. The results demonstrate that, despite no evidence of conditioned responding by group (A+/IO-)A:P, excitability changes in the B photoreceptor that have been correlated with the development of conditioned responding develop normally.

Previous research with vertebrates (Matzel et al. 1988) has called into question the necessity for a stimulus to possess predictive value in order to support associative learning (see also Papini and Bitterman 1990). Rather, a contiguous relationship between two stimuli was proposed to be sufficient to support the formation of an associative memory, whereas the expression of that association may be a function of the stimulus' informational value. Consequently, a failure to respond may be more a function of the response system being examined, rather than an indication of the underlying association. To the extent that conditioning-induced changes in B photoreceptors (i.e., increased excitability) represent a primary cellular correlate of the CS-US association, our results from experiment 2 are consistent with such a conclusion and, in this regard, are not mechanistically compatible with the formulation of Rescorla and Wagner (1972) or Pearce and Hall (1980). Accordingly, these findings are more consistent with theories postulating a contextual modulation of behavioral responding, as opposed to a disruption of the CS-US association (e.g., Gibbon and Balsam 1981; Miller and Schachtman 1985). This interpretation, of course, rests on the assumption that the changes in B-cell excitability represent a necessary and sufficient mechanism for the formation of a CS-US association. This limitation is considerable when one considers that multiple sites of plasticity have been identified in *Hermissenda* following associative conditioning (e.g., Fryszak and Crow 1993; McPhie et al. 1993). It follows then that

blocking may result from a disruption of the associative changes in one or several of these alternative sites of plasticity, regardless of changes in B photoreceptors. Empirically, this counterargument is difficult to disprove in that there could always be another covert site or mechanism of plasticity. Reconciliation of this argument will ultimately rely on more complete sampling of the sites of plasticity in *Hermissenda*, as well as positive identification of the proposed downstream interference process.

The possibility still remains that the behavioral blockade observed in experiments 1 and 2 resulted from a peripheral mechanism working independent of central forms of plasticity. Experiment 3 addressed this issue by monitoring the motor output to the light CS in animals receiving differential contextual conditioning.

Experiment 3

In experiment 2 it was found that the pairing-specific changes in B photoreceptor excitability develop normally in animals for which there is no evidence of a CR at the behavioral level. Experiment 3 was designed to account for this apparent discrepancy between the cellular and behavioral data by utilizing known learning-induced changes in motor system activity as a correlate of behavioral responding.

Several cells and putative motoneurons have been identified in the pedal ganglia of *Hermissenda* that exhibit a sensitivity to light (Jerussi and Alkon 1981; Goh and Alkon 1984; Hodgson and Crow 1992) and whose activity is subject to modification by associative changes in type B photoreceptors (Goh et al. 1985). Goh and Alkon (1984) described the first complete input-output pathway in *Hermissenda* that has since been shown to exhibit conditioning-specific changes following paired light and rotation training (Goh et al. 1985). Briefly, type A photoreceptors excite central visual neurons located in the cerebropleural ganglion (CPG), which in turn monosynaptically excite an identified putative motoneuron (MN1) within the pedal ganglia. The axon of MN1 exits the central nervous system via pedal nerve 1 (P1), which innervates the posterior half of the foot (Richards and Farley 1987). Excitation of MN1 results in the constriction of the ipsilateral, posterior half of the animal's foot and is thought to participate in *Hermissenda's* orientation toward a light source (Goh and Alkon 1984). Associative training results

in a reduction of the light-evoked impulse frequency in MN1 (Goh et al. 1985) and multiunit activity (MUA) in P1 (Richards and Farley 1987), which are thought to participate in the associative reduction of phototaxis observed following pairings of light and rotation. Richards and Farley (1987), investigating MUA (extracellular) in P1, reported high levels of tonic activity in naive dark-adapted preparation. With the onset of a light stimulus, MUA rates increased by 10%–12%. In contrast, following associative conditioning, the light-evoked increase in MUA rate was suppressed for paired animals relative to their random controls.

Experiment 3 was designed to address the working hypothesis that, despite the presence of associative changes in the B photoreceptors, the expression of the association (i.e., suppression of light-induced MN1 activity) is modulated at a more central location in the sensory–motor network. Consequently, there should be no difference in nerve activity between behaviorally blocked animals and their unpaired controls, regardless of the changes in B-photoreceptor excitability. In an attempt to demonstrate a central modulatory locus for contextual blocking, experiment 3 further evaluates two other possible mechanisms that might account for the lack of conditioned responding. First, neuromuscular junctions of invertebrates exhibit considerable plasticity that can directly impact on subsequent changes in behavior (e.g., Wojtowicz et al. 1991, 1994). Second, other studies with invertebrates have demonstrated behavioral changes owing to plastic modifications in the peripheral nervous system (e.g., peripheral motor neurons; for review, see Carew and Sahley 1986). In monitoring motor activity as it exits the nervous system, experiment 3 will enable us to evaluate the extent to which these peripheral mechanisms contribute to behavioral evidence of associative blocking. It must be noted that the training-induced reduction in activity in P1 has been correlated previously with the training-induced suppression of phototaxis in *Hermissenda* (Richards and Farley 1987). This same *reduction* of activity could not easily account for the behavioral index of conditioning used in the present paper (i.e., foot contraction). However, because the conditioning-specific changes in motor output in P1 have been demonstrated previously and because foot contraction and suppression of phototaxis are highly correlated (Matzel et al. 1989), we chose to concentrate our efforts on MUA in P1 in the present paper.

Materials and Methods

SUBJECTS

Twenty-seven naive *Hermissenda*, housed and maintained under the same general description as in experiments 1 and 2, served as subjects in this experiment.

BEHAVIORAL CONDITIONING AND TESTING

The behavioral conditioning apparatus was the same as used in experiment 1. The chemosensory cues were also prepared and maintained in the same manner described previously. All groups were preexposed to the US using the differential conditioning procedure and received CS–US training as described for experiment 2. Twenty-four hours following the final training session, animals were prepared for extracellular electrophysiological analysis. All electrophysiological work was done in a coded manner such that the experimenter was unaware of the animal's treatment history. No behavioral testing was conducted in this experiment.

ELECTROPHYSIOLOGY

Just prior to extracellular recording, nervous systems were surgically isolated and pinned to a glass slide using a single stainless steel insect pin placed across the cerebral commissure and secured with petroleum jelly. Extracellular MUA was recorded during the dark and in response to a 4-sec light stimulus (durations and intensities correspond to the CS used for behavioral conditioning). The severed ends of pedal nerve P1 (Richards and Farley 1987) were drawn into glass suction pipettes with tip diameters of 50–100 μm . Tip diameters were varied to obtain the tightest nerve/electrode junction, thereby increasing the extracellular resistance and maximizing the recorded potentials. Extracellular signals were amplified differentially (WPI model DAM-50; $\times 10,000$ AC) and filtered (low pass 300 Hz; high pass 1 kHz). Data acquisition was accomplished using an analog-to-digital converter (Digidata 1200, Axon Instruments, Inc.) connected to a microcomputer (CompuAdd Corp.) running data acquisition software (Fetchex, Axon Instruments, Inc.). Sampling rates of 2.5 kHz were used for all data acquisition. A typical acquisition protocol consisted of 15 min of dark adaptation followed by 12 sec of continu-

ous data acquisition. The first 4 sec of recording was done in complete darkness and provided an estimate of baseline nerve activity. This interval was followed by a 4-sec light step delivered through a fiber optic bundle. Light responses were induced with an unfiltered white light emitted from a 21.5-V, 150-W (nominal) tungsten halogen projector lamp focused through the fiber optic bundle and modulated to produce an intensity at the nervous system comparable to that used during training. An additional 4 sec of nerve activity was recorded at the termination of the light step to end the protocol. All data were stored on magnetic disk for subsequent off-line analysis.

STATISTICAL ANALYSIS

Digitally acquired data were analyzed using event detection software (Fetchan, Axon Instruments, Inc.). A single event was counted when the signal exceeded the overall mean MUA (i.e., baseline) by 1 s.d. (see also Richards and Farley 1987). Frequency histograms (bin widths = 0.05 sec) were then generated for each animal from these event lists (stat, Axon Instruments, Inc.). These data were then broken into 4-sec groupings and collapsed for each animal into a single score that represented the mean number of recorded events prior to, during, and after light presentation. The data were compared using a two-way repeated measures ANOVA in which factor 1 was the conditioning history of the animals and factor 2 was the time of recording (i.e., pre-, during, or postlight). Additionally, the percent change in the number of recorded events from baseline levels was calculated (mean activity during light/mean activity during premeasure) for each animal and subjected to a one-way ANOVA in which the single factor was conditioning history.

Results and Discussion

In three replications, the extracellular MUA of P1 was monitored during 4-sec dark and light intervals following contextual blocking. Three groups received 3 days of experience with two different contexts (scallop extract or IO) each day. For each group, one context was reinforced by 50 unsignaled presentations of rotation, whereas the second context was unreinforced. Following preexposure training, all groups received a single session (60 trials) of light-rotation

conditioning within the scallop chemosensory context (A) in either a paired or explicitly unpaired fashion. All electrophysiological analyses occurred 24 hr following the final conditioning trial.

Figure 5 presents frequency histograms in which the mean MUA (impulses/0.05 sec) before, during, and after a 4-sec light step are plotted for each group. In concordance with previous work (Richards and Farley 1987), group (IO + /A -)A:P ($n=9$), which was shown in the previous two experiments to exhibit conditioning-specific changes in both light-evoked behavior as well as in characteristics of the B photoreceptor, showed lower levels of light-evoked activity relative to unpaired animals (Fig. 5A). This attenuation is relative to the unpaired animals, group (A + /IO -)A:UP ($n=9$), in which a light-induced increase in nerve activity was observed (Fig. 5B) that was comparable to that typically observed in naive animals (Richards and Farley 1987). Consistent with the observation of behavioral blocking, group (A + /IO -)A:P ($n=8$) failed to exhibit any suppression in light-induced nerve activity. Rather, MUA rates of this group are equivalent to the unpaired control condition.

These data were further quantified by collapsing the data into 4-sec bins, such that a single score was obtained for each animal for recorded events prior to, during, and after light presentation. One-way ANOVA found that the groups did not differ in MUA rates (impulses/4 sec) prior to the onset of the test light, $F(2,23)=0.23$, ns. The data were then normalized by computing the percent change in nerve activity from the prelight measure. Figure 6 presents these data for all three groups, both during (4 sec) and after (4 sec) light presentation. With the onset of light, all three groups exhibited a light-induced increase in activity, although the magnitude of increase was determined by the conditioning history. One-way ANOVA revealed a main effect of conditioning history during the 4-sec light step, $F(2,23)=5.80$, $P<0.01$. Group (A + /IO -)A:UP displayed an ~23% increase in activity and did not differ significantly from blocked animals, group (A + /IO -)A:P, by planned comparison, $F(1,23)=0.48$, ns. In contrast, the light-induced activity of group (IO + /A -)A:P was significantly attenuated relative to groups (A + /IO -)A:P, $F(1,23)=6.14$, $P<0.05$, and (A + /IO -)A:UP, $F(1,23)=9.94$, $P<0.01$.

These findings replicate previous work demonstrating an associative reduction in P1 nerve ac-

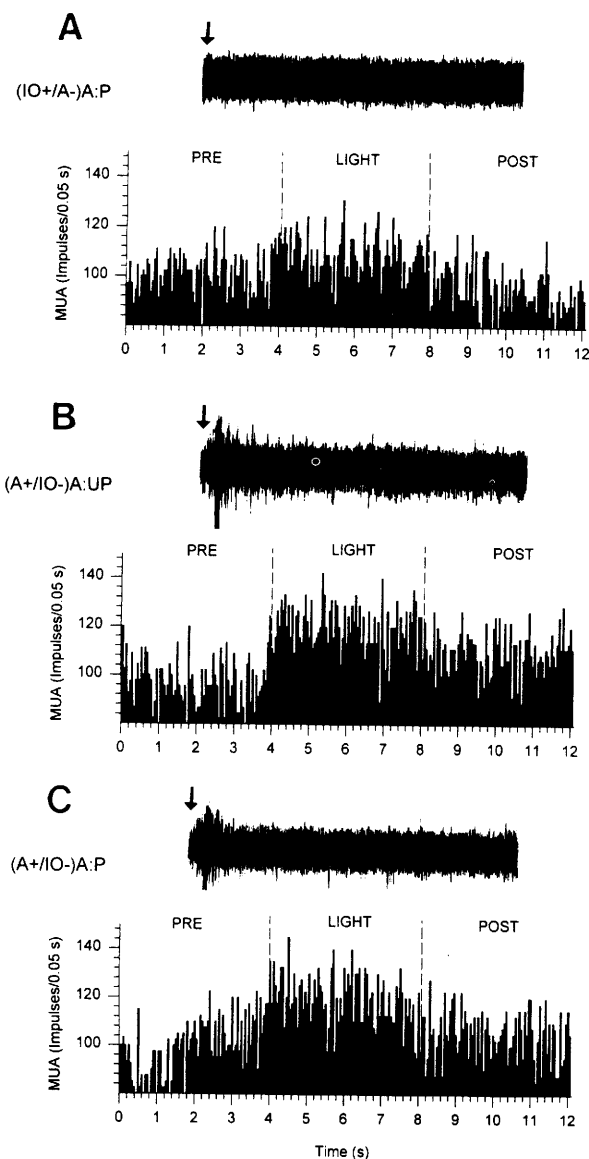


Figure 5: Light-induced MUA following contextual blocking. Pedal nerve 1 was recorded from using an extracellular suction electrode. Histograms are presented representing the mean number of nerve impulses/0.5 sec prior to, during, and after a 4-sec light presentation. Above each histogram is a record obtained from one animal in that group during the 4-sec light presentation (arrow represents light onset). A suppression of the normal light-induced activity was observed in group (IO+/A-)A:P relative to the unpaired control, (A+/IO-)A:UP. In contrast, light-induced nerve activity for group (A+/IO-)A:P did not differ from unpaired animals, a finding that is consistent with their lack of conditioned responding.

tivity following paired light and rotation training (Richards and Farley 1987). Experiment 2 found that group (IO+/A-)A:P, which experienced a

context shift between US preexposure and CS-US conditioning, develops a normal conditioned foot contraction to the light CS, a CR that is known to parallel associative decreases in phototaxis (Matzel et al. 1989). Moreover, it was determined that a context shift between US preexposure and CS-US pairings was sufficient to disrupt contextual blocking of the CS-US association; that is, a normal conditioned response developed. These same animals were found to exhibit a conditioning-induced increase in B-cell excitability that typically accompanies memory formation. The demonstration of attenuated P1 nerve activity in the present experiment is thus consistent with both the primary cellular changes (i.e., B-cell excitability) and the behavioral expression of these changes (e.g., decreased phototaxis) in animals that exhibit normal learning. Additionally, experiment 2 demonstrated the failure of group (A+/IO-)A:P to behaviorally express any CS-US association, despite the evidence of an increase in excitability in the B cell. However, in experiment 3 we found no difference in light-induced nerve activity between group (A+/IO-)A:P and the unpaired control condition, a finding that is consistent with the lack of conditioned responding in each of these groups.

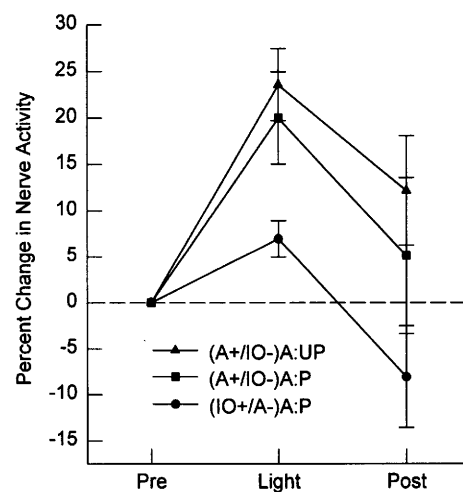


Figure 6: Percent change in light-induced MUA following contextual blocking. Mean data for the percent change in light-induced MUA. Data were calculated by taking the mean MUA (impulses/0.05 sec; see Fig. 5) across 4-sec intervals representing the time just prior to light onset, during light, and after light offset. An associative reduction of light-induced nerve activity was observed in group (IO+/A-)A:P, whereas groups not expressing any conditioned responding [i.e., groups (A+/IO-)A:P and (A+/IO-)A:UP] did not differ in the light responsiveness. Brackets indicate s. e.

In summary, an increase in B-cell excitability was observed in animals that exhibit evidence of behavioral blocking of the CS–US association. Despite the presence of this cellular correlate of association formation, there is a lack of motor output necessary, at least in part, for the expression of a conditioned suppression of phototactic behavior. In its most simplistic form, the input–output pathway mediating this learning can be organized into three stages, that is, the primary receptors (B cells), an interneuronal network, and the motoneurons participating in behavioral output. What follows logically from this simplified reasoning is that the expression of the primary CS–US association is blocked at the level of the interneuronal network. In other words, the context–US association formed during the preexposure phase may result in the modification of neuronal transmission within this interneuronal network. In this regard, blocking may result from a central biasing of the sensory–motor network against the expression of an association with an afferent loci (e.g., the B photoreceptors) and/or the attenuation of central CS–US associations necessary for learning. At present, we cannot distinguish between these two possibilities. It should be noted though that the context–US association does not appear to be maintained at the level of the chemoreceptors or tentacular ganglia, in that all behavioral testing occurred within a neutral chemosensory context where no chemosensory information was available for the activation of these sensory pathways (experiment 1). Moreover, during the assessment of the B-cell excitability and the MUA at the pedal nerve, the nervous system was surgically isolated from the chemoreceptors. Thus the CS–US association must be modulated at a central location relative to the chemosensory apparatus, that is, the interneuronal network. Unfortunately, information concerning this network is limited and little is known concerning synaptic communication within the network itself (see General Discussion, below).

General Discussion

BEHAVIORAL EVIDENCE FOR CONTEXTUAL BLOCKING

In two separate experiments, the contextual blocking effect was investigated in the marine mollusc *Hermisenda* using diffuse chemosensory cues to define the contextual background. Exper-

iment 1 found that US preexposure within a well-defined context was sufficient to block the development of conditioned responding to a light CS when the conditioning of the light occurred within the excitatory context but not when conditioning was conducted in an associatively neutral context. A similar effect was observed in experiment 2 where animals were preexposed to multiple contexts, one of which was paired with un signaled US presentations (i.e., differential contextual conditioning); only the context paired previously with the US was capable of blocking the development of a conditioned response to the light CS. This modulation of learning and/or behavior by an excitatory context extends the work of Rogers et al. (1996), who demonstrated the development of an association between the diffuse chemosensory context and US presentations that occur within that context. Additionally, this work adds to the rapidly growing list of phenomena characteristic of vertebrate learning found in invertebrate animals (Carew and Sahley 1986; Byrne 1987).

CELLULAR CORRELATES OF THE CS–US ASSOCIATION FOLLOWING BLOCKING

Theoretical accounts of the blocking effect differ largely in regard to the nature of the disruption, that is, a modulation of associative strength (Rescorla and Wagner 1972; Pearce and Hall 1980) versus a disruption of behavioral expression (Gibbon and Balsam 1981; Miller and Schachtman 1985). If in fact the medial B photoreceptor was the sole site of plastic change in the *Hermisenda* nervous system following light–rotation pairings, then a failure to express learning-specific changes in the B cell following the blocking procedure would confirm the predictions of Rescorla and Wagner (1972) and/or Pearce and Hall (1980), whereas changes in the B cell (in the absence of a behavioral conditioned response) would be more consistent with Gibbon and Balsam's (1981) and/or Miller and Schachtman's (1986) account of blocking. Although evidence suggests that the B photoreceptor contributes to the conditioned response elicited by light (e.g., Crow and Alkon 1980; Farley et al. 1983; Matzel et al. 1992), numerous sites of plasticity have now been identified (Farey et al. 1990; Fryszak and Crow 1993; McPhie et al. 1993). Consequently, a definitive test of these hypotheses would require sampling from all

of these sites of plasticity (and presumably other, as yet unidentified, sites), as well as a complete understanding of their relative contribution to the conditioned response. Nevertheless, theories that suggest that blocking procedures should result in a learning failure, by inference, suggest that the association should fail to be expressed biophysically. With the appreciation that our analysis is incomplete, experiment 2 tested this latter prediction, based on the assumption that one of the primary storage sites for the light–rotation association was in the medial B photoreceptor. Specifically, following the establishment of behavioral blocking, we investigated associative increases in the excitability of type B photoreceptors.

Based on our sampling of the medial B cell, our results provide tentative support for the suggestion that the lack of conditioned responding reflects a disruption in the expression of the association and not a disruption in the formation of the association at the cellular level. Both paired groups, despite differential behavioral responding, exhibited equivalent increases in B-cell excitability relative to the unpaired control group. Consequently, our findings are more consistent with theoretical formulations that postulate deficits in performance (e.g., Miller and Schachtman 1985), as opposed to associative strength (e.g., Rescorla and Wagner 1972). Acceptance of this conclusion should, of course, be tempered by the evidence for multiple sites of plasticity and/or mechanisms of plasticity (e.g., Schuman and Clark 1994) in this system. These caveats notwithstanding, the data reported here are consistent with behavioral evidence in vertebrate species that suggests that blocking may be a failure to express a normal CS–US association (for review, see Delamater and LoLordo 1991). Moreover, it has been reported (Neuenschwander-El Massioui et al. 1991) that procedures that produce behavioral blocking of a tone–shock association in rats result in a normal increase in tone-induced cellular activity in hippocampal CA3 field. These results are analogous to those reported here, that is, a normal increase in B-cell excitability despite behavioral blocking. Of course, the results of Neuenschwander-El Massioui et al. (1991) are subject to the same caveat as ours, namely, that although their data are consistent with theoretical accounts of blocking that assume the formation of a normal CS–US association, it cannot be taken as conclusive evidence for such a process given that the role of the hippocampus in this type of learning is uncertain. Moreover, in

at least one instance, neurophysiological correlates of blocking have been reported that appear to be in accordance with the predictions of Rescorla and Wagner (1972). Sears and Steinmetz (1991) report that unit activity within the inferior olive (US) decreases on paired trials across the course of classical eye-blink conditioning in rabbits. The diminished olivary activity is thought to result from inhibitory projections from the red nucleus that feed back CR-related information onto the US-related pathway (Donegan et al. 1989; Thompson 1990). Consequently, it has been proposed (Thompson 1990; Sears and Steinmetz 1991) that activation of the red nucleus by a pretrained CS should attenuate US processing in the inferior olive, thus diminishing its ability to support subsequent conditioning (i.e., blocking).

Despite an increase in B-cell excitability after the behavioral blocking procedure used here, the motor output ostensibly necessary for one form of the CR indicative of a light–rotation association is blocked. For instance, the normal suppression of P1 nerve activity in response to light that follows light–rotation pairings was not evident in animals that exhibited behavioral blocking. Given that these animals exhibit an equivalent increase in B-photoreceptor excitability relative to unblocked animals, we can speculate on several possibilities. First, it is conceivable that the changes in B-cell excitability that accompany conditioning are not directly related to “memory” formation or the generation of the conditioned response. This possibility seems unlikely given prior evidence that such changes are sufficient to induce behavioral changes analogous to those observed after conditioning (e.g., Farley et al. 1983) and correlate well with the magnitude of the conditioned response in normally conditioned animals (e.g., Matzel et al. 1992). A more likely possibility is that following a blocking procedure, the expression of the light–rotation association is modulated or blocked at some point downstream to the primary sites of plasticity in the B photoreceptors. The most likely site for this modulatory interaction is a central network of identified interneurons. These interneurons are known to receive inputs from chemosensory, visual, and vestibular sensory receptors and are therefore well suited to serve as a site for plastic modifications coding for chemosensory contextual stimuli, as well as a behavioral gating mechanism for the light–rotation association. At present, it must be stressed that blocking may result from a modulation by the interneuronal net-

work of the association “stored” in the B photoreceptors or may reflect the failure to develop as yet unidentified forms of training-induced plastic modifications within the interneuronal network. We cannot distinguish between these two possibilities, the former of which is consistent with “comparison” accounts of blocking (e.g., Gibbon and Balsam 1981; Miller and Schachtman 1985), whereas the latter is consistent with “competition” accounts (e.g., Rescorla and Wagner 1972; Pearce and Hall 1980).

IMPLICATIONS FOR NETWORK MODELS OF LEARNING IN *HERMISSENDA*

Several central features of contemporary learning theories must be discussed to fully appreciate the implications of the present results. Importantly, all theories of associative learning described here share the common assumption that contextual blocking requires the formation of an association between contextual stimuli and the same US ultimately employed in CS–US pairings. These models differ with regard to their assumptions concerning the formation of an association between the CS and US in the excitatory context. So-called competition models assume that the CS–US association is not formed owing to the fact that the context already is a good predictor of the US and thus supersedes the CS in the competition for associative strength (e.g., Rescorla and Wagner 1972; Pearce and Hall 1980). In contrast, comparison models (e.g., Gibbon and Balsam 1981; Miller and Schachtman 1985) assume that CS–US contiguity is sufficient for the formation of associations and that an excitatory training context blocks the *expression* of that learning (for assessment of the behavioral implications of these models, see Durlach 1989; Delamater and LoLordo 1991). For these later theories to account for the expression of blocking at the time of testing, it must be assumed that a third association is formed during conditioning trials, specifically, between the CS and the context in which it is trained, thus providing for context specificity (i.e., so that an excitatory context other than the CS training context does not block the expression of an association at the time of testing). The provision that an association is formed between all stimuli present on a conditioning trial and, specifically, between the CS and its training context is an integral feature of modern theoretical accounts of learning (e.g.,

Mackintosh 1985; Wagner and Brandon 1989; Miller et al. 1991; Holland 1993; Capaldi and Neath 1995).

Assuming that contextual blocking in *Hermis-senda* is mechanistically similar to that in vertebrate species (as the behavioral data seems to indicate), any attempt to describe this process in *Hermis-senda* at the neural level must account for the capacity for the animal to form a context–US (rotation) association as well as an association between the CS (light) and the US (rotation). Moreover, to account for the contextual specificity of the blocking that we have observed, we must assume that the CS (light) becomes associated with the excitatory context in which CS–US training occurs. At present, our understanding of the neural network of *Hermis-senda* is at too preliminary a stage to accurately describe such a complex sequence of interactions. It should also be noted that even a complete understanding of the synaptic interactions within a network is usually insufficient to account for motor output in the absence of a complete understanding of the dynamics of each cell in the circuit (Marder 1994; Weimann and Marder 1994). Nevertheless, the results reported here suggest that prior models of network interactions in *Hermis-senda* (e.g., Lederhendler et al. 1986) are inadequate to account in total for the expression of the association between light and rotation. Moreover, these models do not contain any provision for blocking (CS–US contiguity is sufficient for learning and conditioned response generation), central modulation of a learned response, or for contextual modulation of discrete associations.

Perhaps the most important and most speculative implication of the results reported here is the issue of interneuronal plasticity that has been assumed, though not demonstrated. Clearly, this interneuronal network represents one of the main processing areas for both simple and more complex associations in *Hermis-senda*, as it does in other systems (e.g., Cleary et al. 1995). Despite the appreciation of this requirement, we remain largely ignorant in regard to the interneuronal network, the synaptic interactions therein, and their capacity to exhibit learning-induced forms of plasticity.

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