Generalization of Habituation and Intrinsic Sensitization in the Leech

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Abstract

Using the shortening reflex of the medicinal leech *Hirudo medicinalis* we examined stimulus generalization of habituation learning. Preparations received mechanosensory stimulus at two positions on the leech body wall, one site used to carry out habituation training and a second novel site to test for generalization of habituation. After training, the specific mechanosensory neurons activated by each stimulus were assessed using intracellular recordings. As expected, the closer the two sites were to each other, the greater the degree of generalization of habituation at the novel site and the more sensory cells were shared. However, a form of behavioral facilitation was observed at the trained site that resembled behavioral sensitization, but differed from the standard sensitization process in several respects. (1) Facilitation was induced by stimulation of the novel site before habituation training at the trained site, although the stimulus intensity at the novel site was equivalent to the training stimuli and was not the strong, noxious stimuli that normally induce sensitization. (2) The magnitude of the facilitating effect was proportional to the proximity of the novel and trained stimulation sites. (3) Although behavior at the trained site was facilitated, behavior at the novel site was habituated, indicating that the induced behavioral facilitation did not generalize throughout the animal, as normally occurs during sensitization, but was limited to a single stimulus-response pathway.

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Introduction

A critical feature of learning in any animal is the ability to discriminate between learned and novel stimuli. Failure to discriminate between a trained (S_T) versus a novel (S_N) stimulus could result in an inappropriate response. The degree to which this generalization occurs depends on how similar S_T and S_N stimuli are to each other (Mackintosh 1974). It is suggested that this is determined at a physiological level by the number neural elements shared by S_T and S_N , both at afferent and integration levels. In addition, generalization can be affected by the type of learning that the animal undergoes. For example, it is believed that associative learning and habituation involve modification of a behavioral response to a specific stimulus, therefore, the level of generalization of the learned behavior to other novel stimuli is low (Mackintosh 1974; Stopfer et al. 1996). In contrast, sensitization is thought to alter behavior throughout the animal, in effect, generalizing to many if not all stimulus-response pathways (Carew et al. 1971; Boulis and Sahley 1988; Davis 1989; Lockery and Kristan 1991).

The whole-body shortening reflex of the medicinal leech *Hirudo medicinalis* has been a useful system for studying associative and nonassociative forms of learning (Sahley 1995). This defensive behavior may be elicited by either photic or mechanosensory stimulation that generates a coordinated contraction of all the leech body segments (Lockery et al. 1985; Boulis and Sahley 1988). Mechanosensory stimuli in the leech are detected by at least three classes of sensory neurons. In order of most to least sensitive, they are the touch (T), pressure (P), and nociceptive (N) cells. Every segmental ganglion contains three T, two P, and two N cells innervating each side of the body. The receptive fields of the T and P cells innervate a discrete region of skin along the medial (ventral/dorsal) axis of the body segment (Nicholls and Baylor 1968;

Yau 1976) providing the leech with positional information about the stimulation site (Lockery and Kristan 1990a; Lewis and Kristan 1998).

Whole-body shortening requires stimulation of the body wall sufficient to activate two P cells with adjacent receptive fields and associated T cells (Shaw and Kristan 1995). This stimulation results in contraction of the longitudinal muscles starting at the site of stimulation as a result of activity from the L-motor neuron and the dorsal and ventral excitatory (DE and VE, respectively) motor neurons in each body segment (Fig. 1) (Stuart 1970; Shaw and Kristan 1995). The reflex spreads throughout the body as a result of activity from an unknown intersegmental neural pathway and a chain of interconnected interneurons called the S cell. The S cell, although not critical to the whole-body shortening reflex (Sahley et al. 1994), does contribute to the behavior by virtue of its excitatory input to the L cell (Gardener-Medwin et al. 1973; Shaw and Kristan 1995).

Given the requirement for whole-body short-

Figure 1: Simplified diagram of the neural circuit mediating the leech whole-body shortening reflex. Mechanosensory input to the skin is transduced by T, P, N cells that stimulate activity in the motor neurons, which induce contraction of the longitudinal muscles. The signal to shorten is carried to motor units throughout the body by the S cell and a second, unidentified interneuron. The presence of this latter cell has been inferred by Shaw and Kristan (1995) and all inputs to and outputs from this cell (dashed lines) are hypothetical. Solid rectangles connecting adjacent S cells represent electrical synapses.

ening to activate multiple mechanosensory cells across two receptive fields, we asked how similar must two mechanosensory stimuli be in terms of position on the body wall before habituation training at one site generalizes to whole-body shortening initiated at a second novel site. Using a combination of behavioral and electrophysiological techniques, we examined how habituation generalized between two stimulus-response pathways. In addition, these experiments revealed a form of behavioral facilitation, resembling sensitization, that did not generalize throughout the entire animal, but was specific to a single stimulus-response pathway.

Materials and Methods

QUASI-INTACT PREPARATION

Each leech was anesthetized by cooling at 4°C and all dissections were carried out in normal leech saline (Muller et al. 1981) maintained at 4°C. A longitudinal incision was made down the dorsal midline of the leech between segments 3 and 9. The next two segments (10 and 11) were left intact and the ventral nerve cord (VNC) was dissected out from segments 12 to 13 (Fig. 2). The preparation was then pinned onto a Sylgard-lined dish ventral side up so that the body wall portion of the preparation (segments 3–9) formed a flat sheet and the dish was filled with leech saline. Pairs of 0.2-mm Teflon-coated silver wire (Medwire) electrodes were implanted in the skin and bared at the point of contact with the skin to stimulate discrete areas of the leech body wall. One pair of electrodes was designated S_T (trained site) and would be used for habituation training. A second pair of electrodes was designated S_N (novel site) and would be used to test for generalization of habituation. Both electrode pairs were implanted in the same body segment (segment 8), but in differing positions so that they activated different mechanosensory receptive fields within that segment. In addition, a window was cut in the ventral midline of the body wall to expose the segmental ganglia containing the sensory cells that would be activated by either stimulation site (segments 7–9). A third pair of electrodes (S_{DH}) was implanted in segment 4 and was used to deliver dishabituating stimuli to the preparation.

The intact portion of the preparation was connected to an isometric tension transducer (Grass Instruments) to record the magnitude of the short-

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Figure 2: Diagram of the quasi-intact preparation used in habituation generalization experiments. Electroshock stimulus was delivered using silver wire electrodes (not shown) implanted in the body wall portion of the preparation and behavior was measured using a tension transducer attached to the intact portion of the preparation. A window was opened in the body wall to expose the ganglia from segments 7 to 9. Neural activity in the VNC was recorded using a suction electrode.

ening response. A suction electrode on the VNC was used to record activity from the S cell, which generates the largest action potential in extracellular recordings of the VNC (Bagnoli et al. 1972). For some of the preparations examined, a branch of the dorsal posterior segmental nerve (DP:B1) that contains the axon for the L-motor neuron (Ort et al. 1974) was exposed and activity from that cell was recorded using a second suction electrode. Extracellular recordings were made using a Grass P/5 A.C. preamplifier. Intracellular recordings of mechanosensory cells and motor neurons were made using sharp glass microelectrodes (15–20 M Ω resistance, Sutter Instruments P-80 micropipette puller) filled with 3 M potassium acetate and an Axoclamp 2A amplifier (Axon Instruments). Data from all recording devices was viewed on a storage oscilloscope (Tektronix) and converted for digital storage using a TL-1 analog/digital interface and pClamp 6.21 data acquisition program (Axon Instruments).

After setup, the preparation was allowed to recover for ~30 min before proceeding with the

next stage of the experiment. The leech saline was replaced manually using a Pasteur pipette at ~15-min intervals throughout all subsequent experimental stages. Both the leech saline and the room in which all experiments were conducted was maintained at ~24°C.

STIMULATION PARAMETERS

The shortening response was stimulated by the delivery of a single 2-msec duration, capacity-coupled electroshock to the skin with the stimulation parameters controlled by a Grass S88 Stimulator and SIU5 Stimulus Isolation Unit. The intensity of the shock was determined by the minimum level that could elicit reliably a robust shortening response (5- to 15-nA range). This differs from previous experiments carried out with semi-intact leeches where the stimulus intensity was set within a predetermined range (Boulis and Sahley 1988; Ehrlich et al. 1992; Sahley et al. 1994). In those experiments one stimulus range was used during habituation training that activated only T and P cells, whereas a second, stronger stimulus range that activated T, P, and N cells was used to deliver sensitizing and dishabituating stimuli (Sahley et al. 1994). In this more reduced preparation the stimulus intensity necessary just to elicit a shortening response for habituation training was strong enough to activate N as well as T and P cells (see Results). The behavioral thresholds were determined separately for each of the stimulation sites (S_T , S_N , and the dishabituating site) and were set so that each site elicited a shortening response of approximately the same magnitude. After determination of the stimulus levels at all three sites, the preparation was allowed to rest for ~15–20 min before training commenced.

TRAINING PROCEDURE

The training protocol used in these experiments is summarized in Figure 3. Before training at the S_T site the baseline shortening response at the S_N site was determined [baseline = average response across three trials, 2-min intertrial interval (ITI)]. Two minutes after the last S_N baseline trial, habituation training was carried out at the S_T site using the protocol outline by Sahley et al. (1994; 20 trials, one pulse per trial, 2-min ITI). Data from 20 training trials were averaged into four and five trial blocks. The first block (consisting of trials 1–5) was

Figure 3: Diagram summarizing the training procedures used to test generalization of habituation.

used as the baseline for the subsequent responses elicited at S_T . After habituation training at S_T , the behavioral response at the S_N site was probed for generalization of habituation (as with the baseline, the average response across three trials). The preparation was then dishabituated at the S_{DH} site by delivering two trains of 10 shocks (2-msec pulse duration, 10 Hz, 2-min ITI). After delivery of the dishabituating stimuli the behavioral responses at both S_T and S_N were retested (five trials each at S_T and S_N , 2-min ITI).

After habituation and dishabituation training, the level of sensory overlap between S_T and S_N was determined. Sensory overlap was measured as a proportion of preparations in which a mechanosensory cell was stimulated by both the S_T and S_N sites divided by the number of times that that cell was activated by one site or the other. For example, the G8 lateral T cell in the ventral/dorsal group was stimulated by S_T or S_N in nine different preparations and of these was stimulated by both sites in six preparations, yielding a sensory overlap value of 67%. Activity from the T, P, and N cells in segmental ganglia 7–9 was recorded intracellularly, while the S_T or S_N site was stimulated, providing a quantitative measure of the level similarity between the S_T and S_N at the level of the primary afferents. These data were used to confirm that the positioning of S_T and S_N electrodes progressively closer to each other did result in greater levels of sensory overlap.

Experiments were carried out on four treatment groups and one control group. Each treatment group had a characteristic placement of the S_T and S_N pairs of electrodes and these electrode pairs were placed progressively closer across treatment groups (see Figs. 6–9, below, for electrode placement). Because the approximate distribution of mechanosensory receptive fields in the leech is known, it is possible to know a priori where on the skin to activate a certain set of sensory cells (e.g., dorsal vs. ventral afferents). Nevertheless, the mechanosensory cells activated by each skin electrode pair were confirmed by intracellular recordings after training. The treatment groups used in these experiments were, in order of level of sensory overlap, ventral/dorsal ($n = 11$) < ventral/lateral ($n = 11$) < posterior/anterior ($n = 10$) < overlapping (S_T and S_N electrode pairs were placed as close to each other as possible; $n = 10$). Preparations in the control group ($n = 12$) had electrodes implanted for habituation training and delivering dishabituation stimuli only and therefore, did not receive any extra stimulation associated with testing behavior at the S_N site.

STATISTICS

For data analysis, the behavioral data from each trial block were normalized to the initial baseline response. In the case of S_T , the habituation and dishabituation trial blocks (trial block 2–5) were normalized to the first habituation training trial block. In the case of the S_N , data from the habituation generalization probe and dishabituation trial blocks (trial blocks 4 and 5, respectively) were normalized to the baseline trial block taken before habituation training at S_T . Behavioral data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures. Analysis was carried out using the Statistica (Statsoft, Inc.) statistics software package.

Results

PROPERTIES OF THE QUASI-INTACT PREPARATION

Behavioral changes during habituation and dishabituation training in the leech quasi-intact preparation were identical to that observed in intact and semi-intact preparations used by Sahley and co-workers, based on results obtained from the control group (Fig. 4; Boulis and Sahley 1988; Ehrlich et al. 1992; Sahley et al. 1994). Repeated electro-

Figure 4: Changes in whole-body shortening behavior in the quasi-intact preparation from the control group during habituation training (trial blocks 1–4) and after the delivery of a strong dishabituating stimulus (trial block 5). Data have been normalized to the first trial block and have been presented as the mean $\pm$ standard error. (DHS) Dishabituating stimulus.

shock stimulation at S_T resulted in a decrease in the magnitude of whole-body shortening (trial blocks 1–4) and this response decrement could be reversed in trial block 5 after the presentation of a noxious stimulus at S_{DH} .

Activity from individual neurons known to participate in shortening are shown in Figure 5. The electroshock stimulus elicited a train of action potentials in the T cells, but only a single action potential in the P and N cells (Fig. 5A). Figure 5B provides an example of the activity in the S-interneuron and L-motorneuron during a stimulated shortening episode. The level of L-cell activity (mean $\pm$ S.E. = 3.31 ± 1.41 ; $n = 5$) was comparable, albeit somewhat lower, to what has been observed in other reduced preparations (Shaw and Kristan 1995) and the presence of L-cell activity six segments away, along with activity in the S cell, confirms that whole-body shortening was in fact the behavior being activated. Another observation from this trace involves the tight coupling of excitatory postsynaptic potentials (EPSP) and action potentials in the L cell with S-cell action potentials (Fig. 5B). The S cell is known to make an excitatory electrical synapse onto the L cell (Magni and Pelli-grino 1978) and the S cell may contribute to trans-

Figure 5: Sensory and motor properties of whole-body shortening in the quasi-intact preparation. (A) Intracellular recordings of activity evoked by stimulus sufficient to induce shortening in these preparations from T (top), P (middle), and N cells (bottom). (B) Simultaneous recording of stimulus-evoked neural and behavioral activity. The top two traces are extracellular and intracellular recordings respectively of the L-motor neuron in segment 14. The corresponding behavior recorded from a tension transducer is shown in the second from the bottom trace. The bottom trace is an extracellular record of S cell activity recorded from the VNC posterior of segment 15. The notation next to the vertical calibration bar in B corresponds to the L cell intracellular recording and the shortening response, respectively.

mitting shortening-inducing stimuli throughout the leech body (Shaw and Kristan 1995). Although Figure 5B demonstrates S-cell input onto an L cell several segments from the stimulus site, it should be noted that not all preparations exhibited a one-for-one correspondence between S- and L-cell impulses.

SENSORY OVERLAP

The placement of the electrode pairs in each of the treatment groups was effective at generating increasing degrees of sensory overlap in the ventral/dorsal, ventral/lateral, posterior/anterior, and overlapping groups (Figs. 6–9). In preparations where the S_T and S_N electrodes were relatively far apart (e.g., the ventral/dorsal group; Fig. 6) the level of sensory overlap was low except in sensory cells whose receptive fields bordered the areas stimulated by both S_T and S_N . As the electrode pairs were placed closer together, the level of sensory overlap increased in all of the afferent cells regard-

less of whether the electrode pairs were placed bilaterally (e.g., the ventral/lateral group; Fig. 7), or unilaterally (e.g., the posterior/anterior and overlapping groups; Figs. 8 and 9). This pattern was seen both in the segment where the electrodes were implanted (G8) and in mechanosensory cells from adjacent segments (G7 and G9). The pattern of sensory overlap across the four treatment groups indicates that stimuli used in these experiments are capable of activating a distinct set of mechanosensory neurons depending on the placement of the electrodes. Although N cells were also tested in this manner, they are not included in this analysis as their receptive fields span the entire segment and as a result were stimulated by both S_T and S_N sites regardless of their placement.

BEHAVIORAL DATA

As seen in Figures 6–9 a second form of behavioral plasticity emerged besides habituation. In

Figure 6: Sensory overlap and behavioral data from the ventral/dorsal group. (Left) The receptive fields of the touch (T) and pressure (P) mechanosensory neurons across three segments are diagrammatically shown and the shading in each receptive field indicates the percentage of times that the corresponding sensory neuron was activated by both stimulation sites in the same preparation (values for each shade of gray are shown at the figure bottom). The subscripts D, L, and V stand for dorsal, lateral, and ventral respectively. The + and – symbols indicate the placement of the implanted electrodes for each site. (Right) The behavioral response elicited by the S_T site (○) is habituated after repeated stimulations (blocks 1–4) and dishabituates after the delivery of a noxious stimulus several segments away (block 5). The shortening reflex elicited by the S_N site (△) is unchanged from the baseline indicating no generalization of habituation. The control data (●) are from control preparations where no S_N site was used (see Fig. 3) and have been plotted in Figs. 4–7 as a comparison to the treatment groups.

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Figure 7: Sensory overlap and behavioral data from the ventral/lateral group. (Left) Same as Fig. 6. (Right) The S_T behavior does habituate by trial block 4, but the response at block 3 is substantially greater than in the control group. The behavior at the S_N site has decreased significantly from the initial response, indicating generalization of habituation between these two sites.

preparations where there was little sensory overlap between S_T and S_N , such as in the ventral/dorsal group, there was a substantial decrease in

the behavioral response elicited at S_T after habituation training (Fig. 6) and the level of habituation was virtually identical to that observed in the con-

Figure 8: Sensory overlap and behavioral data from the posterior/anterior group. (Left) Same as Fig. 6. (Right) The shortening reflex elicited by S_T does not habituate after repeated stimulations. The reflex elicited by S_N does decrease significantly relative to its baseline indicating that the operational habituation training applied to S_T did generalize to S_N .

Figure 9: Sensory overlap and behavioral data from the overlapping group. (Left) Same as Fig. 6. (Right) The shortening reflex elicited by S_T does not habituate after repeated stimulations. The reflex elicited by S_N does decrease significantly relative to its baseline indicating that the operational habituation training applied to S_T did generalize to S_N .

trial group (see Fig. 4). However, in preparations from the posterior/anterior and overlapping groups, where the level of sensory S_T/S_N overlap was high, the magnitude of the shortening reflex elicited at S_T was unchanged from the baseline after habituation training (Figs. 8 and 9). Preparations from the treatment group that had an intermediate level of sensory overlap, the ventral/lateral group, were also intermediate in terms of the change in S_T -elicited shortening after habituation training (Fig. 7). Although this group did habituate (see difference between trial blocks 1 and 4), the shortening response level during trial block 3 was greater than in the corresponding trial blocks in groups where normal habituation occurred.

It appears that stimulation of S_N before habituation training at S_T induced a behavioral facilitation process that prevented normal habituation at S_T when there was sufficient sensory overlap between the two stimulation sites. (Stimulation of S_N before S_T habituation training was done to obtain a baseline response level for the shortening reflex.) This conclusion was confirmed by a two-way ANOVA that revealed a statistically significant interaction effect of treatment group (placement of S_N electrodes relative to S_T) and habituation training on S_T -elicited behavior (behavioral response of trials 1–4) ($F_{\text{inter}} = 2.14$, $P \leq 0.05$, $df = 12$). Although there was not a significant effect attrib-

able to treatment group alone ($F_{\text{treat}} = 2.48$, $P \geq 0.05$, $df = 4$), there was a statistically significant effect of training trial ($F_{\text{trial}} = 7.29$, $P \leq 0.001$, $df = 3$). Posthoc analysis (LSD test) indicated that there was a significant decrease in the behavioral responses from trial block 4 relative to those at trial block 1 in the control, ventral/dorsal, and ventral/lateral groups ($P \leq 0.05$), whereas no significant change in behavior was detected in the posterior/anterior and overlapping groups ($P > 0.05$). A two-way ANOVA that examined the effects of treatment group and dishabitating stimuli (trial block 4 vs. 5) detected a significant trial effect ($F_{\text{trial}} = 6.80$, $P \leq 0.05$, $df = 1$), but no effect attributable to treatment ($F_{\text{treat}} = 1.35$, $P > 0.05$, $df = 4$) or treatment/trial interaction ($F_{\text{inter}} = 0.62$, $P > 0.05$, $df = 4$). To summarize, the data clearly show that the ability of a preparation to carry out habituation in this training paradigm was dependent on the proximity of the S_N and S_T sites.

Generalization of habituation from S_T to S_N was observed in all but the ventral/dorsal treatment groups (Figs. 6 to 9). Two-way ANOVA indicated that there was a significant decrease in the magnitude of the S_N response at trial block 4 relative to the initial baseline ($F_{\text{trial}} = 19.78$, $P \leq 0.0001$, $df = 1$). Post-hoc analysis (LSD) showed a statistically significant decrease in the S_N response in the ventral/lateral, anterior/posterior, and overlapping

groups, but not the ventral/dorsal group, after habituation training at S_T . This change in the behavior elicited at S_N was dependent on the relative positions of the S_T and S_N electrodes ($F_{\text{treat}} = 2.88$, $P \leq 0.05$, $df = 3$; $F_{\text{inter}} = 2.88$, $P \leq 0.05$, $df = 3$). However, generalization of habituation at S_N was apparently not dependent on the actual habituation of S_T -elicited behavior, but only on the operational habituation at S_T as no habituation at this site was observed in the posterior/anterior and overlapping groups. Testing for dishabituation at the S_N site, a two-way ANOVA indicated a significant increase in shortening between trial blocks 4 and 5 ($F_{\text{trial}} = 4.94$, $P \leq 0.05$, $df = 1$). The changes in trial block 4–5 behavior were not affected by the placement of the S_T and S_N electrodes ($F_{\text{treat}} = 2.00$, $P > 0.05$, $df = 3$; $F_{\text{inter}} = 1.09$, $P > 0.05$, $df = 3$). An additional group of preparations were tested to confirm that observed changes in shortening were in fact attributable to habituation training. Quasi-intact preparations stimulated at a single site on the skin, at the beginning and end of a 40-min period to reproduce the time course of habituation training without the repetitive stimulations, showed no changes in behavior (paired $t = 1.50$, $P > 0.05$, $df = 3$; data not shown).

To assess the duration of this behavioral facilitation, the overlapping experiment shown in Figure 9 was repeated, except that a 10-min delay period was interposed between the S_N baseline trials and the start of S_T habituation training (data not shown). The S_T response from this delay group ($n = 12$) did not differ from the overlapping group, S_T -elicited behavior did not decrease during habituation training, indicating that facilitating effect had not yet decayed ($F_{\text{trial}} = 1.11$, $P > 0.05$, $df = 1$; $F_{\text{treat}} = 0.02$, $P > 0.05$, $df = 3$; $F_{\text{inter}} = 0.29$, $P > 0.05$, $df = 3$). Generalization of habituation at S_N was also observed in the delay group and did not differ from the overlapping group in this respect either ($F_{\text{trial}} = 21.74$, $P \leq 0.001$, $df = 1$; $F_{\text{treat}} = 0.66$, $P > 0.05$, $df = 1$; $F_{\text{inter}} = 0.66$, $P > 0.05$, $df = 1$).

Discussion

Different types of learning exhibit different levels stimulus discrimination as is illustrated by the degree to which trained stimuli generalize to a subsequently presented novel stimulus. Classical conditioning and habituation are relatively stimulus specific and generalize only to very similar

novel stimuli (Mackintosh 1974; Stopfer et al. 1996; but see Krasne and Teshiba 1995), whereas sensitization generalizes to nearly all potential novel stimuli (Carew et al. 1971; Boulis and Sahley 1988; Davis 1989; Lockery and Kristan 1991). Using a quasi-intact preparation of the medicinal leech the level of generalization of habituation of the whole-body shortening response between two positionally distinct skin stimulation sites was observed. As expected, stimulation sites that were closer to each other and shared a larger proportion of the same mechanosensory neurons exhibited generalization of habituation. Unexpectedly, the training protocol used to observe generalization of habituation at S_N produced a second, facilitating form of behavioral plasticity at S_T . This facilitating process showed a high degree of stimulus specificity because it was observed at S_T at the same time that habituation was present in S_N -elicited behavior.

PROPERTIES OF THE QUASI-INTACT PREPARATION

The behavior observed in the quasi-intact preparation generally conforms with the whole-body shortening observed in intact and semi-intact leeches. One difference is the greater stimulus intensities needed to initiate whole-body shortening. In other preparations, stimulation of T and P cells across two receptive fields is sufficient to elicit whole-body shortening (Boulis and Sahley 1988; Shaw and Kristan 1995), whereas in quasi-intact preparations, activation of the N cells was required as well. The reason for the greater stimulation may be attributable to the more reduced nature of the preparation. Alternatively, the reason could involve inhibitory input from proprioceptive elements of the nervous system attributable to the pinning of the preparation, which prevents a substantial portion of the animal from moving. Stretch receptors are present in the leech skin (Blackshaw and Thompson 1988) and such proprioceptive input is known to modify leech swimming behavior (C.G. Hocker and W.O. Friesen; X. Yu and W.O. Friesen; both unpubl.). Nevertheless, we believe that the observed behavior can be classified as whole-body shortening and does not differ substantially from the behavior that would be observed in an intact leech because (1) N cells can be activated by stimuli that induce shortening (Shaw and Kristan 1995); (2) the number and type of sensory neurons activated by the stimulus and the level of activity by the L-motorneuron and the S-interneuron

resemble the neural activity observed in other preparations; and (3) control group behavior during habituation and dishabituation training is similar to behavioral results obtained from intact and semi-intact leeches (Boulis and Sahley 1988; Sahley et al. 1994).

GENERALIZATION OF HABITUATION

The level of generalization of habituation in the leech whole-body shortening response was dependent on how close the S_N electrodes were to the S_T electrodes. This was in large part attributable to the proportion of mechanosensory cells shared by both stimulation sites, based on the experimental groups in which generalization was observed (ventral/lateral, posterior/anterior, and overlapping groups, but not the ventral/dorsal group). This is consistent with experiments in *Aplysia* (Stopfer et al. 1996) where generalization of habituation would occur between two nearby training sites, but not between two sites on opposite sides of the animal. In contrast, in the crayfish escape response, habituation could generalize between two bilaterally separated stimulation sites that shared no primary afferents (Krasne and Teshiba 1995). In the leech it appears that a moderate level of sensory overlap was sufficient to support generalization of habituation given that generalization was observed in a group that had a lower proportion of shared sensory cells (ventral/lateral) as compared to the other two training groups. Thus, generalization may not be attributable to general sensory overlap, but rather to overlap of a specific cell type, namely the P cells. Whole-body shortening requires stimulation across two P-cell receptive fields (Shaw and Kristan 1995) and when two stimulation sites shared at least one P cell, generalization could take place. This is supported by experiments in another defensive behavior in the leech, local bending, which have shown that the level of activity in different P cells can communicate effectively the position of a mechanosensory stimulus as a result of the connections between the P cells and a network of interneurons that are part of local bending circuit (Lockery and Kristan 1990a,b; Lewis and Kristan 1998). It is possible that positional information of stimuli inducing whole-body shortening is processed by a similar sensory processing network.

In the posterior/anterior and overlapping groups, where generalization of habituation was observed, habituation was observed only at the S_N

site and not at the S_T site. The reason for this lack of habituation at S_T appears to involve a novel mechanism of behavioral facilitation, which will be discussed more fully below. Nevertheless, the data show that the neural processes underlying habituation cannot exclusively involve a depression in signaling along monosynaptic pathways between sensory to motor or interneuron connections (Hawkins et al. 1993). This model would predict that any time generalization of habituation was observed at S_N as a result of sharing mechanosensory cells with S_T , habituation should also be seen at S_T . This was not the case in our experiments indicating that habituation is mediated, at least in part, by heterosynaptic pathways. Krasne and Teshiba (1995) have shown that habituation of the crayfish escape response is largely dependent on a descending inhibitory input outside of the direct stimulus-response pathway. Goldberg and Lukowiak (1984) observed a decrease in the *Aplysia* siphon withdrawal response after habituation of the gill withdrawal reflex, indicating the presence of a heterosynaptic inhibition during habituation. Furthermore, experiments on habituation of the tail and siphon withdrawal reflexes in *Aplysia* showed an increase in heterosynaptic eppsp's at the sensory-to-motor synapse instead of homosynaptic depression (Stopfer and Carew 1996), providing additional evidence for the presence of a heterosynaptic process for habituation in invertebrates.

An alternative interpretation of the changes in S_N -induced shortening is that habituation transferred from the S_T to the S_N stimulus-response pathway. This provides a potential explanation for how S_N -induced shortening could undergo habituation, whereas behavior at S_T remained unchanged (as in the posterior/anterior and overlapping groups). As mentioned above, Goldberg and Lukowiak (1984) observed transfer of habituation of the gill withdrawal reflex to the siphon withdrawal response, although these two structures do not appear to share any sensory units [in contrast to the results of Stopfer and Carew (1996)]. However, we still believe that generalization of habituation from S_T to S_N remains the more likely explanation. Habituation at S_N did depend on increasing sensory overlap with S_T and habituation of both S_T and S_N was observed in one of the experimental groups (ventral/lateral). Although generalization of habituation at S_N in the absence of habituation at S_T is surprising, we believe that this can be explained by the behavioral facilitatory process described below.

AN INTRINSIC PROCESS CONTRIBUTING TO SENSITIZATION

Interestingly, in groups where both sites shared all or nearly all the same mechanosensory cells, two opposite forms of behavioral plasticity, habituation at S_N and a facilitating process at S_T , were observed. Furthermore the extent of habituation at S_T was attributable to the proximity of the S_T electrodes to the S_N electrodes. That is habituation at S_T was not affected when there was little sensory overlap between S_N and S_T , but when the level of sensory overlap was high, habituation was prevented at S_T in such a way that did not affect generalization of habituation to S_N .

Preparations with little sensory overlap between S_T and S_N , such as the ventral/dorsal group, underwent normal habituation at S_T , whereas those with substantial overlap, the anterior/posterior and overlapping groups, did not habituate. The group with an intermediate level of sensory overlap (ventral/lateral) did undergo habituation at S_T , but here too the behavioral pattern during habituation training was different from that observed in the ventral/dorsal or control groups. The pattern of S_T behavior during habituation training in the overlapping and posterior/anterior groups resembles sensitization of the leech whole-body shortening reflex (Boulis and Sahley 1988; Ehrlich et al. 1992; Sahley et al. 1994). Sensitization of this behavior is characterized, in part, by a failure to habituate to repeated stimulations and is induced by the delivery of a substantially stronger electroshock stimulus delivered several segments away from the test stimulus. In the current experiments we observed a similar phenomenon, a failure of S_T -elicited shortening to habituate, although no explicit sensitizing stimulus had been presented. Instead these preparations received stimulation within the same segment (the S_N site) before training of a magnitude that was similar to the stimulus used at S_T site.

Although phenomenologically similar, this form of behavioral facilitation is distinct from traditional sensitization. Sensitization is normally induced by a strong stimulus that differs in terms of modality or intensity from the test stimulus used to elicit the behavior of interest (Boulis and Sahley 1988; Davis 1989). However, in our case the stimuli applied at S_N were approximately equivalent both in magnitude and position to S_T and would not be expected to induce sensitization. Second, the facilitating effect of a sensitizing stimu-

lus is the same regardless of where that stimulus is applied. In these experiments, the strength of the observed facilitating effect was determined by the proximity of the S_N site to the S_T site (see Figs. 6–9). Third, the facilitating effect of traditional sensitization generalizes to all subsequent behavioral responses, something that was not seen with the S_N -induced behavioral facilitation. In groups where habituation at S_T did not occur (posterior/anterior and overlapping), the S_N -elicited behavior still underwent habituation as a result of the habituation training at S_T (see trial block 4 in Figs. 8 and 9). However, the behavioral facilitation observed at S_T was not observed at S_N indicating that generalization is not characteristic of this form of sensitization.

Davis and File (1984) have stated that habituation and sensitization can occur as a result of both intrinsic and extrinsic neurophysiological processes. An intrinsic process would involve modulation that occurred within the stimulus–response pathway of the behavior of interest, whereas an extrinsic process would involve modulation from neural components outside of that pathway. Habituation is thought traditionally to occur by way of an intrinsic mechanism (Castellucci et al. 1970; Thompson et al. 1973; but see Krasne and Teshiba 1995). Sensitization, on the other hand, is generally believed to be an extrinsic process involving the activity of modulatory interneurons that are activated by a sensitizing stimulus, but not part of a stimulus–response neural pathway being tested (Thompson et al. 1973; Castellucci and Kandel 1976; Byrne et al. 1978; Hawkins et al. 1981). Evidence of an extrinsic mechanism contributing to habituation has been observed in the crayfish (Krasne and Teshiba 1995), *Aplysia* (Goldberg and Lukowiak 1984; Stopfer and Carew 1996), and the data presented here, but no indication of the intrinsic sensitization process hypothesized by Davis and File (1984) has been reported. It would appear that the behavioral facilitation that we have seen in the leech-shortening response represents an observable form of intrinsic sensitization. The sensitization effect is specific to a single stimulus–response pathway (S_T) is induced by stimulation of a site on the leech body wall (S_N) that shares most of the same sensory neurons as S_T , and can be observed at S_T while S_N is in a habituated state. It should be stressed that intrinsic sensitization is not, by itself, a form of nonassociative learning. Intrinsic sensitization is a behavioral facilitating process that contributes (along with extrinsic mechanisms)

to the nonassociative learning known as sensitization.

There is a precedent for behavioral facilitation occurring without presentation of an explicit sensitizing stimulus. Incremental (Razran 1971) or wind-up sensitization (Hinde 1970) is a form of behavioral facilitation generated during the initial stages of habituation training (Davis and Wagner 1969; Groves et al. 1970; Lockery and Kristan 1991). This form of behavioral facilitation occurs as a result of activating a single stimulus-response pathway and not attributable to the presentation of a different sensitizing stimulus. It has been suggested that such behavioral facilitation reflects an initial incremental process that decays because of an independent, slower developing decremental or habituating process, as predicted by dual-process theory (Thompson et al. 1973). It is possible that, as with the intrinsic sensitization observed here, the behavioral facilitation that occurs during incremental sensitization is the result of an intrinsic sensitizing process activated in the absence of the extrinsic processes that would normally also contribute to sensitization.

Recently, it has been recognized that neuromodulation can occur intrinsically as well as extrinsically (for review, see Katz and Frost 1996). Extrinsic modulation is thought to be ubiquitous and involves the modification of a neural circuit through the release of neuromodulators from neurons not part of the circuit being activated (Harris-Warrick 1991). Intrinsic modulation is believed to be the result of neuromodulators released by neurons within the circuit itself (Katz and Frost 1996). Modulatory transmitters are coreleased with standard neurotransmitters by a neuron within a given circuit, altering the response of a target neuron or muscle to signals from the modulatory cell or from another neuron within the circuit. Intrinsic neuromodulation has been observed to modify behavior at the neuromuscular junction (Cropper et al. 1987, 1990) and central pattern generator (CPG) level (Whim and Lloyd 1989; Cropper et al. 1990; Meyrand et al. 1991; Weiss et al. 1992; Katz and Frost 1995, 1996). Intrinsic modulation is precisely the sort of physiological mechanism that Davis and File (1984) described as likely to mediate intrinsic sensitization. Facilitation of a specific stimulus-response circuit requires that the modulatory process occurs within the activated circuit. Extrinsic modulation, on the other hand, would facilitate inactive as well as active stimulus-response pathways. A simplified, hypothetical model of how in-

trinsic and extrinsic modulatory processes would differentially contribute to sensitization is presented in Figure 10.

Now that a protocol has been developed to behaviorally isolate the processes that mediate intrinsic and extrinsic sensitization, it is necessary to characterize the neurophysiological mechanisms that distinguish intrinsic from extrinsic sensitization. Extrinsic sensitization of the leech whole-body shortening reflex is dependent on serotonin (5HT) released from the Retzius cell and the serotonergic system can be selectively lesioned by treating animals with the toxin 5,7-dihydroxytryptamine (5,7-DHT) (Ehrlich et al. 1992). Leeches treated with 5,7-DHT are unable to undergo sensi-

Figure 10: Intrinsic versus extrinsic mechanisms of sensitization. On the basis of activation patterns of the sensory units, each stimulation site has a unique stimulus-response pathway (SR-). In this diagram all three stimuli converge to generate the same behavior, but stimulus III could alternatively produce a completely different behavior from stimuli I and II. (A) Stimulation at II induces intrinsic sensitization in I (indicated by the dotted line and shaded circle) because there is considerable sensory overlap between I and II. Intrinsic sensitization is not induced in III because there is no sensory overlap between II and III. (B) Extrinsic sensitization is induced by a qualitatively different stimulus (sensitizing stimulus) that has no sensory units in common with stimulus I, II, or III. The sensitization produced by this process affects all three stimulus-response pathways (indicated by the dotted lines and shaded circles).

tization, but the ability to carry out whole-body shortening is not affected. It is reasonable to hypothesize that 5HT dependence is one feature that distinguishes extrinsic from intrinsic sensitization and that 5HT-depleted animals will still be able to undergo intrinsic sensitization if this facilitatory process is not dependent on Retzius cell-released 5HT. These experiments are currently underway. Although an intrinsic modulatory process has yet to be identified in the leech whole-body shortening reflex, a potential candidate is the S-interneuron because (1) the S cell is active during whole-body shortening and contributes to plasticity of this behavior (Sahley et al. 1994; Shaw and Kristan 1995; Modney et al. 1997); and (2) the S cell contains the modulatory peptide myomodulin (Keating and Sahley 1996). Because it is possible to disrupt the S cell chain by selectively cutting the S cell axon (Mason and Muller 1996; Modney et al. 1997), an interesting experiment would be to induce intrinsic sensitization in S cell axotomized leeches to determine whether intrinsic sensitization is dependent on an intact chain of S cells.

CONCLUSIONS

The data presented demonstrate that habituation and sensitization, supposedly simple forms of learning, are in fact quite complex. Habituation of the leech whole-body shortening reflex is mediated, at least in part, by heterosynaptic processes and generalization of habituation can occur when two stimuli share at least one of the two P cells required to initiate the behavior. In addition, an intrinsic facilitating process that contributes to sensitization was observed that potentiated the behavior elicited from a single stimulus-response pathway. This intrinsic sensitizing process was specific enough to prevent habituation at the site of training, even when habituation could be observed at a nearby untrained site as a result of generalization. Understanding the neural basis of intrinsic sensitization could help clarify the physiological processes of associative learning. Extrinsic sensitization processes have been shown to contribute to associative learning both in the leech (Sahley 1994) and in *Aplysia* (Hawkins et al. 1993), but the stimulus discrimination properties of associative learning and sensitization are very different. In associative learning, the learned behavioral response is fairly specific to the trained stimulus, therefore the level of generalization is relatively low. Extrinsic sensitization, however, alters the behavioral re-

sponse to nearly all subsequent stimuli and therefore, the level of generalization is quite high. It is possible that intrinsic sensitization provides a facilitating process specific to a single stimulus-response pathway that contributes to the associative learning. This does not discount the potential role of extrinsic sensitization in associative learning, but instead suggests that there are both intrinsic and extrinsic facilitating processes that contribute to sensitization and excitatory conditioning.

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References

- Bagnoli, P., M. Brunelli, and F. Magni. 1972. A fast conducting pathway in the central nervous system of the leech *Hirudo medicinalis*. *Arch. Ital. Biol.* **110**: 35–51.
- Blackshaw, S.E. and S.W. Thompson. 1988. Hyperpolarizing responses to stretch in sensory neurons innervating the leech body wall muscle. *J. Physiol.* **396**: 121–137.
- Boulis, N. and C. Sahley. 1988. A behavioral analysis of habituation and sensitization of shortening in the semi-intact leech. *J. Neurosci.* **8**: 4621–4627.
- Byrne, J.H., V.F. Castellucci, and E.R. Kandel. 1978. Contribution of individual mechanoreceptor neurons mediating defensive gill-withdrawal in *Aplysia*. *J. Neurophys.* **41**: 418–431.
- Carew, T.J., V.F. Castellucci, and E.R. Kandel. 1971. An analysis of dishabituation and sensitization of the gill-withdrawal reflex in *Aplysia*. *Intern. J. Neurosci.* **2**: 79–98.
- Castellucci, V.F. and E.R. Kandel. 1976. Presynaptic facilitation as a mechanism for behavioral sensitization in *Aplysia*. *Science* **194**: 1176–1178.
- Castellucci, V.F., H. Pinsker, I. Kupfermann, and E.R. Kandel. 1970. Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. *Science* **167**: 1745–1748.
- Cropper, E.C., P.E. Lloyd, W. Reed, R. Tenenbaum, I. Kupfermann, and K.R. Weiss. 1987. Multiple neuropeptides in cholinergic motor neurons of *Aplysia*. Evidence for modulation intrinsic to the motor circuit. *Proc. Natl. Acad. Sci.* **84**: 3486–3490.

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- Cropper, E.C., M.W. Miller, F.S. Vilim, R. Tenenbaum, I. Kupfermann, and K.R. Weiss. 1990. Buccalin is present in the cholinergic motor neuron B16 *Aplysia* and it depresses accessory radula closer muscle contractions evoked by stimulation of B16. *Brain Res.* **512**: 175–179.
- Davis, M. 1989. Sensitization of the acoustic startle response by footshock. *Behav. Neurosci.* **103**: 495–503.
- Davis, M. and S.E. File. 1984. Intrinsic and extrinsic mechanisms of habituation and sensitization: Implications for the design and analysis of experiments. In *Habituation, sensitization, and behavior* (ed. H.V.S. Peeke and L.F. Petrinovich), pp. 287–323. Academic Press, New York, NY.
- Davis, M. and A.R. Wagner. 1969. Habituation of the startle response under an incremental sequence of stimulus intensities. *J. Comp. Physiol. Psychol.* **67**: 486–492.
- Ehrlich, J., N. Boulis, T. Karrer, and C.L. Sahley. 1992. Differential effects of serotonin depletion on sensitization and dishabituation. *J. Neurobiol.* **23**: 270–279.
- Gardner-Medwin, A.R., J.K. Jansen, and T. Taxt. 1973. The “giant” axon of the leech. *Acta Physiol. Scand.* **87**: 30–31.
- Goldberg, J.I. and K. Lukowiak. 1984. Transfer of habituation in *Aplysia*: Contribution of heterosynaptic pathways in habituation of the gill-withdrawal reflex. *J. Neurobiol.* **15**: 395–411.
- Groves, P.M., D. Lee, and R.F. Thompson. 1970. Effects of stimulus frequency and intensity on habituation and sensitization in acute spinal cat. *Physiol. Behav.* **4**: 383–388.
- Harris-Warrick, R.M. 1991. Modulation of neural networks for behavior. *Ann. Rev. Neurosci.* **14**: 39–57.
- Hawkins, R.D., V.F. Castellucci, and E.R. Kandel. 1981. Interneurons involved in mediation and modulation of gill-withdrawal reflex in *Aplysia*. II. Identified neurons produce heterosynaptic facilitation contributing to behavioral sensitization. *J. Neurophys.* **45**: 315–328.
- Hawkins, R.D., E.R. Kandel, and S.A. Siegelbaum. 1993. Learning to modulate transmitter release: Themes and variations in synaptic plasticity. *Ann. Rev. Neurosci.* **16**: 625–665.
- Hinde, R.A. 1970. Behavioral habituation. In *Short-term changes in neural activity and behavior* (ed. G. Horn and R.A. Hinde), pp. 3–40. Cambridge University Press, London, UK.
- Katz, P.S. and W.N. Frost. 1995. Intrinsic neuromodulation in the *Tritonia* swim CPG: Serotonin mediates both neuromodulation and neurotransmission by the dorsal swim interneurons. *J. Neurophys.* **74**: 2281–2294.
- . 1996. Intrinsic neuromodulation: Altering neuronal circuits from within. *Trends Neurosci.* **19**: 54–61.
- Keating, H.H. and C.L. Sahley. 1996. Localization of the myomodulin-like immunoreactivity in the leech CNS. *J. Neurobiol.* **30**: 374–384.
- Krasne, F.G. and T.M. Teshiba. 1995. Habituation of an invertebrate escape reflex due to modulation by higher centers rather than local events. *Proc. Natl. Acad. Sci.* **92**: 3362–3366.
- Lewis, J.E. and W.B. Kristan. 1998. A neuronal network for computing population vectors in the leech. *Nature* **391**: 76.
- Lockery, S.R. and W.B.J. Kristan. 1990a. Distributed processing of sensory information in the leech. I. Input-output relations of the local bending reflex. *J. Neurosci.* **10**: 1811–1815.
- . 1990b. Distributed processing of sensory information in the leech. II. Identification of interneurons contributing to the local bending reflex. *J. Neurosci.* **10**: 1816–1829.
- . 1991. Two forms of sensitization of the local bending reflex of the medicinal leech. *J. Comp. Physiol. A* **168**: 165–177.
- Lockery, S.R., J.N. Rawlins, and J.A. Gray. 1985. Habituation of the shortening reflex in the medicinal leech. *Behav. Neurosci.* **99**: 333–341.
- Mackintosh, N.J. 1974. Generalization. In *The psychology of animal learning*, pp. 485–542. Academic Press, New York, NY.
- Magni, F. and M. Pelligrino. 1978. Neural mechanisms underlying the segmental and generalized cord shortening reflexes in the leech. *J. Comp. Physiol. A. Sens. Neural Behav. Physiol.* **124**: 339–351.
- Mason, A. and K.J. Muller. 1996. Accurate synapse regeneration despite ablation of the distal axon segment. *Eur. J. Neurosci.* **8**: 11–20.
- Meyrand, P., J. Simmers, and M. Moullins. 1991. Construction of a pattern-generating circuit with neurons of different networks. *Nature* **351**: 60–63.
- Modney, B.K., C.L. Sahley, and K.J. Muller. 1997. Regeneration of a central synapse restores nonassociative learning. *J. Neurosci.* **17**: 6478–6482.
- Muller, K.J., J.G. Nicholls, and G.S. Stent. 1981. *Neurobiology of the leech*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Nicholls, J.G. and D.A. Baylor. 1968. Specific modalities and receptive fields of sensory neurons in CNS of the leech. *J. Neurophysiol. (Bethesda)* **31**: 740–756.
- Ort, C.A., W.B.J. Kristan, and G.S. Stent. 1974. Neuronal control of swimming in the medicinal leech. II. Identification and connections of motor neurons. *J. Comp. Physiol.* **94**: 121–154.
- Razran, G. 1971. *Mind in evolution: An east-west synthesis*

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of learned behavior and cognition. Houghton Mifflin Company, Boston, MA.

Sahley, C.L. 1994. Serotonin depletion impairs but does not eliminate classical conditioning in the leech, *Hirudo medicinalis*. *Behav. Neurosci.* **108**: 1043–1052.

———. 1995. What we have learned from the study of learning in the leech. *J. Neurobiol.* **27**: 434–445.

Sahley, C.L., B.K. Modney, N.M. Boulis, and K.J. Muller. 1994. The S cell: An interneuron essential for sensitization and full dishabituation of leech shortening. *J. Neurosci.* **14**: 6715–6721.

Shaw, B.K. and W.B.J. Kristan. 1995. The whole-body shortening reflex of the medicinal leech: Motor pattern, sensory basis, and interneuronal pathways. *J. Comp. Physiol.* **177**: 667–681.

Stopfer, M. and T.J. Carew. 1996. Heterosynaptic facilitation of tail sensory neuron synaptic transmission during habituation in tail-induced tail and siphon withdrawal reflexes of *Aplysia*. *J. Neurosci.* **16**: 4933–4948.

Stopfer, M., X. Chen, Y. Tai, G.S. Huang, and T.J. Carew. 1996. Site specificity of short-term and long-term habituation in the tail-elicited siphon withdrawal reflex of *Aplysia*. *J. Neurosci.* **16**: 4923–4932.

Stuart, A.E. 1970. Physiological and morphological properties of motoneurons in the central nervous system of the leech. *J. Physiol.* **209**: 627–646.

Thompson, R.F., P.M. Groves, T.J. Teyler, and R.A. Roemer. 1973. A dual-process theory of habituation: Theory and behavior. In *Habituation* (ed. H.V.S. Peeke and M.J. Herz), pp. 239–271. Academic Press, Orlando, FL.

Weiss, K.R., V. Brezina, E.C. Cropper, S.L. Hooper, M.W. Miller, W.C. Probst, F.S. Vilim, and I. Kupfermann. 1992. Peptidergic co-transmission in *Aplysia*: Functional implications for rhythmic behavior. *Experientia* **48**: 456–463.

Whim, M.D. and P.E. Lloyd. 1989. Frequency-dependent release of peptide cotransmission from identified motor neurons in *Aplysia*. *Proc. Natl. Acad. Sci.* **86**: 9034–9038.

Yau, K. 1976. Receptive fields, geometry and conduction block of sensory neurones in the central nervous system of the leech. *J. Physiol.* **263**: 513–538.

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