A Quantal Analysis of the Synaptic Depression Underlying Habituation of the Gill-Withdrawal Reflex in *Aplysia*

(synaptic plasticity/behavior)

VINCENT F. CASTELLUCCI AND ERIC R. KANDEL

Division of Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, N.Y. 10032; and Department of Physiology, New York University School of Medicine, 550 First Avenue, New York, N.Y. 10016

Contributed by Eric R. Kandel, October 10, 1974

ABSTRACT Habituation, one of the simplest behavioral paradigms for studying memory, has recently been examined on the cellular level in the gill-withdrawal reflex in the mollusc *Aplysia* and in the escape response in crayfish. In both cases short-term habituation involved a decrease in excitatory synaptic transmission at the synapses between the sensory neurons and their central target cells. To analyze the mechanisms of the synaptic depression in *Aplysia*, we applied a quantal analysis to synaptic transmission between the sensory and motor neurons of the gill-withdrawal reflex. Our results indicate that short-term habituation results from a presynaptic mechanism: a decrease in the number of transmitter quanta released per impulse. The sensitivity of the postsynaptic receptor remains unaltered.

Habituation, the decrease in a behavioral response to repeated presentation of a novel stimulus, is the most ubiquitous behavioral modification found in animals and man and one of the simplest paradigms for probing memory mechanisms (1-5). Habituation in different response systems and in different animals probably cannot be explained by a single neuronal mechanism. But in animals having a well-differentiated central nervous system, certain types of habituation share similar parametric features and time course, making it likely that in these cases a restricted family of mechanisms is involved. Thus certain reflex responses of vertebrates and higher invertebrates undergo short-term habituation lasting minutes and hours following a single training session of 10 to 15 stimuli, but repeated training sessions can produce long-term habituation that lasts weeks. The acquisition of long-term habituation is sensitive to the pattern of stimulation. Spaced training is more effective in producing the long-term effects than is massed training. Finally, immediate restoration of a habituated response (dishabituation) can be produced by stimulating another pathway (2, 6).

Because habituation can be studied in higher invertebrates and simple vertebrate systems, it is the only behavioral modification that has been successfully studied on the cellular level. In the gill-withdrawal reflex in the mollusc *Aplysia* (7) and in the escape response in crayfish (8), short-term habituation has been found to involve a similar cellular locus and a common synaptic change. There is a decrease in excitatory synaptic transmission (homosynaptic depression) at the central synapses made by the sensory neurons on their central target cells. Synaptic depression, although at a different locus, may also underlie habituation of the flexion reflex in vertebrates (9).

Abbreviations: EPSP, excitatory postsynaptic potential; I.S.I., interstimulus interval; S.N., sensory neuron.

The mechanism producing the synaptic depression is not known. It could be either a presynaptic change in transmitter release or a postsynaptic change in receptor responsiveness. To distinguish between these alternatives a quantal analysis is required. With rare exception (10, 11) this has been difficult to achieve in central neurons because they receive many synaptic inputs, making the analysis of spontaneous release (12) difficult, and because the evoked quantal size is usually small (10-100 μ V) in relation to the background noise and to the synaptic bombardment produced by neural activity.

Using a high divalent cation solution to reduce neuronal activity, electrical filtering to reduce noise, and computer averaging for signal recognition, we have been able to apply a quantal analysis to synaptic transmission between the sensory and motor neurons of the gill-withdrawal reflex in *Aplysia* and to analyze the synaptic depression underlying habituation. Our results indicate that short-term habituation results from a presynaptic decrease in the number of transmitter quanta released per impulse. The sensitivity of the postsynaptic receptor remains unaltered.

METHODS

Abdominal ganglia were isolated from *Aplysia californica* weighing 80-200 g. Analyses were carried out at 20-23° on the excitatory postsynaptic potentials produced by stimulating intracellularly single sensory neurons (7, 13). Single electrodes connected to a bridge circuit were used to stimulate and record from sensory cells; low resistance (less than 5 M Ω) double electrodes were used for the follower cells (usually gill motor cell L7, with some additional data on siphon motor cell LBS-3 and interneuron L16; see ref. 14 for nomenclature). The signals from the follower cells were fed to a cathode follower and then to a dc and a high gain ac amplifier (Tektronix no. 2A61). To minimize baseline noise, the bandwidth of the ac amplifier used was set between 0.6 Hz and 60 Hz. The filtering reduced postsynaptic potential amplitude by about 15%. Data were tape recorded (Hewlett-Packard no. 3960) and displayed on a Brush Recorder (no. 440). Normal solutions of artificial sea water contained 55 mM Mg⁺⁺, 11 mM Ca⁺⁺. When cations were substituted, osmolarity was maintained by deletion of NaCl. Solutions were buffered (pH 7.6) with Tris·HCl.

RESULTS

Synaptic Depression Underlying Habituation of the Gill-Withdrawal Reflex. A weak or moderate intensity stimulus applied to the siphon of *Aplysia* produces a gill-withdrawal reflex that is controlled by the abdominal ganglion [Fig. 1A; (15)]. With 10 to 15 repeated stimuli at 10- to 60-sec intervals

FIG. 1. Synaptic depression at the synapse between mechanoreceptor neurons and motor neurons. (A) Diagram of the abdominal ganglion of *Aplysia* showing schematic innervation of gill and siphon and illustrating simultaneous recording from gill motor neuron (L7) and a mechanoreceptor sensory neuron. (B) Criteria for monosynapticity of the synaptic connection between motor cell (L7) and sensory neuron (S.N.). Independence of synaptic latency on level of transmitter release. (B₁) Comparison of synaptic latency with normal and low release. Superimposed records from the same pair of neurons obtained first (normal release) in normal artificial sea water (55 mM Mg⁺⁺, 11 mM Ca⁺⁺); and then (low release) in a solution that depresses release (165 mM Mg⁺⁺, 8 mM Ca⁺⁺). (B₂) Comparison of synaptic latency with control and increased release. Superimposed records from a pair of neurons before (control) and 10 min after (high release) the amplitude and duration of the presynaptic spike of the sensory neuron were increased by intracellular injection of tetraethylammonium chloride (TEA, 3 M) through the recording electrode. (C) Synaptic depression of the monosynaptic EPSP produced in motor cell L7 by stimulation of a single sensory neuron in high divalent cation solution (138 mM Mg⁺⁺, 62 mM Ca⁺⁺). Interstimulus interval (I.S.I.) 10 sec. Two series of 15 stimuli each were presented with a rest of 15 min in between. The rest led to partial recovery, but a slight build-up of the depression is evident in the second run.

the reflex habituates (16). The neural circuit of this reflex contains six identified motor neurons, a cluster of 24 sensory neurons, and three identified interneurons (13, 15). Studies of the cellular correlates of habituation have shown that tactile stimulation of the siphon skin initiates a complex excitatory postsynaptic potential (EPSP), produced largely by direct synaptic connections from the sensory cells (7, 13), that discharges the motor cells and causes gill contraction. Habituation produces a progressive decrease in the complex EPSP (17). Analyses of the various components of the reflex during short-term habituation (including measurements of the threshold and input conductance of the motor cell) indicate that the critical change occurs at the synapses between the sensory cell and the motor neurons and interneurons (refs. 7 and 17, and manuscript in preparation). With repeated stimulation, the elementary EPSP produced by the sensory neurons decreases in amplitude (Fig. 1C), paralleling the time course of behavioral habituation (16).

Monosynapticity of the Connections between Sensory Cells and Motor Neurons. To analyze the mechanism of the depression in this synaptic potential, it was essential to determine that the connections between sensory and motor cells are monosynaptic. To assess monosynapticity we have used the follow-

ing criteria (7, 18–20). (1) *One-for-one following.* At normal levels of transmitter release every action potential in the sensory neuron produces an elementary EPSP in the follower cell. (2) *Short and constant latency.* At 20–23° the latency between the peak of the presynaptic spike in the sensory neuron and the onset of EPSP in the follower cell is 5–10 msec (Fig. 1B). The latency did not change in solutions containing high (2.6 to 3.0 × normal) concentrations of divalent cations that increase the threshold of neurons and reduce the likelihood of an interneuron's being fired (21, 22). (3) *Independence of latency on amount of transmitter released.* The latency of the EPSP remained unaltered when release was enhanced (Fig. 1B) by increasing the duration of presynaptic spike (intracellular injection of tetraethylammonium into the sensory cell) and reduced (20-fold) by increasing the Mg⁺⁺ content (165 mM) of the artificial sea water. These criteria are consistent with a monosynaptic connection.

Quantal Fluctuations under Conditions of Low Transmitter Release. We carried out a quantal analysis to examine the mechanism of the synaptic depression. This analysis is based on the finding that the transmitter at chemical synapses is released in multimolecular packets (quanta) and that any synaptic potential is made up of an integral number of these quanta (12).

With reduced transmitter output (increasing Mg⁺⁺ to 165 mM and reducing Ca⁺⁺ to 8 mM),^a repetitive stimulation of the sensory neuron produced marked fluctuations in the amplitude of the EPSP, in an apparently quantal fashion, and occasional failures (Fig. 2). Amplitude histograms of 30 to 100 consecutive responses (Fig. 3A–D) revealed a multimodal distribution, with the mean of each subsequent peak being an integral multiple of the unit peak. To improve the signal-to-noise ratio^b and insure that we correctly identified failures, we averaged all putative failures, using a PDP8-E computer, so as to determine whether a smaller occult unit EPSP had gone unrecognized (Fig. 3E₁). Averaging did not reveal a smaller, occult unit EPSP^c; the averaged failures were

^a This solution contains 2.6 × normal divalent cation concentration and, therefore, also serves to increase the threshold of cells, dramatically reducing the spontaneous impulse activity in the ganglion. The high Mg⁺⁺ concentration does not seem to produce a significant effect on the receptor. The kinetics of decrement are roughly similar in normal sea water and in high Mg⁺⁺ solution.

^b We did not examine spontaneous miniature potentials because of the background noise, much of it probably produced by the spontaneous transmitter release from presynaptic cells that synapse on follower cells. The noise level dropped by 50–70 % on withdrawal of the electrode from the cell. Moreover, doubling the osmolarity of the solution with sucrose produced showers of small depolarizations and hyperpolarizations, suggesting a massive recruitment of miniature potentials. In three instances we made amplitude histograms of the depolarizations by collecting the excursion of the baseline that had a similar rise time to the evoked EPSPs. The peak of a unit size correlated with the unit peak of the evoked EPSPs in one case, but in the two other cases many peaks were observed and the results were ambiguous.

^c In two experiments we also have used a blind procedure. The histograms of the original EPSPs were independently graphed by a naive observer. In both cases the $\bar{q}$ and failure values obtained were similar to ours. We also did amplitude histograms of 200 samples of the background fluctuation taken at a fixed interval after a spike and several seconds after the EPSP was over. These histograms contained no trends and no peaks corresponding to the peaks of the evoked EPSPs.

FIG. 2. Quantal fluctuations accompanying synaptic depression at a low level of release (165 mM Mg^{++} , 8 mM Ca^{++}). Samples of successive EPSPs evoked in L7 by a series of 200 consecutive intracellular stimuli to the sensory neuron at 10-sec intervals. Total membrane potential shift during the series was less than 1 mV. Samples are taken from consecutive responses in three different plateau regions defined in the text. The first region (see examples 5 to 8) shows marked amplitude fluctuations and no failures (see Fig. 3A for details). In regions 2 and 3 there is a progressive increase in the number of failures (arrows; see also Fig. 3A). Number beside each pair of traces refers to the stimulus number in the series. The amplitude histograms of the EPSPs (Fig. 3A to D) were obtained by measuring the magnitude of the response in time intervals (window) between the peak of the pre-synaptic spike and the peak of the first EPSP (1).

indistinguishable from the averaged background fluctuations. We also averaged the unit EPSP and found that it had a similar configuration to the average of the first few large evoked EPSPs (Fig. 3E₂), indicating that one can also successfully recognize by eye the unit quantal signal. These findings, and the further documentation below, demonstrate the feasibility of a quantal analysis at this synapse.

Quantal Analysis of Depression at Low Release. When the mean probability (p) of a quantum's being released is low and the available mean population of quanta (n) is large, transmitter release can be approximated by a Poisson distribution. An assumption of this distribution is that the number of quanta released by an action potential (m = the mean quantum content) is $m = n \times p$. The average amplitude ($\bar{E}$) of an EPSP is then $\bar{E} = m \times \bar{q}$ where $\bar{q}$ is the mean size of the unit EPSP. Measurements of m and $\bar{q}$ provide the most direct method for distinguishing between pre- and postsynaptic processes. The m value indicates the amount of transmitter released by the presynaptic terminal and a constant $\bar{q}$ value indicates an unchanging sensitivity of the postsynaptic receptor.^d

Assuming a Poisson distribution, we estimated quantal content (m) and quantal size ($\bar{q}$) with reduced transmitter

^d A reduction in $\bar{q}$ value is more difficult to evaluate. Given normal filling it indicates a decrease in postsynaptic responsiveness. But some pharmacological agents (e.g., hemicholinium) seem to interfere with normal "vesicular filling" and also cause $\bar{q}$ to decrease.

FIG. 3. Amplitude histograms and computer averages of unitary EPSPs and failures during synaptic depression. (Parts A-D) Four sets of amplitude histograms of successive EPSPs during the synaptic depression accompanying 144 to 219 consecutive intracellular stimuli to the sensory neuron. The regions refer to periods of stable responses as described in the text. The first response peak was assumed to be the amplitude of a unitary EPSP (q_1 , as defined in the text). The successive peaks were roughly integral multiples of the initial peak. From the value of m_1 obtained from these amplitude histograms a predicted curve (broken line) was generated assuming a Poisson distribution and a coefficient of variation for q_1 of 30%. Roman numerals and vertical arrows refer to the successive multiples of the estimated unit potential. Arrows on the ordinate refer to the predicted number of failures. Arrows on the abscissa refer to the mode. The mode shifts to the left (more failures) with repeated stimulation. N = number of stimuli; the two values in parentheses refer to the first and last EPSP of the plateau region (A and C, cell #2 of Table 1, I.S.I. 10 and 60 sec; B, cell #1, I.S.I. 10 sec; D, cell #3, I.S.I. 60 sec). (E) Computer averages of the unitary EPSPs and failures during the synaptic depression accompanying 200 consecutive stimuli (cell #2, part A). (E₁) Data were averaged on a PDP8-E computer. Using tape delay loops, the peak of the presynaptic spike triggered the averaging procedure; the first five EPSPs of the series were averaged to determine average EPSP and to establish the interval window (see Fig. 2). Failures ($N = 21$) and unit EPSPs were averaged separately ($N = 21$). The background noise ($N = 21$) was averaged 2 sec before the occurrence of a presynaptic spike. The failure average resembled the background noise average; there was no time-locked depolarization. (E₂) Time course of average EPSP and average unit. The averaged EPSP and the unit average of part E₁ were matched for amplitude and superimposed photographically to illustrate the similarity in their time course.

output in 11 series of experiments on various follower cells (in six different preparations). The evaluation of a Poisson distribution requires many responses. Because of the plasticity of these synapses it is difficult to obtain a large number of evoked EPSPs at the low frequencies (once every 10 min) that do not

TABLE 1. Quantal analysis of synaptic depression

Cell and amplitude 1st EPSP	Trials	m_1	m_2	m_3	$\bar{q}_1$ (μ V)	$\bar{q}_2$ (μ V)	$\bar{q}_3$ (μ V)
I.S.I. = 10 sec							
Cell #1	1-9*	7.3	—	9.8	34	—	25.4
(L7)	10-39	3.9	2.7	4.4	37.5	49.6	30.2
406 μ V	40-109	1.7	1.5	1.8	34	39.3	33.5
	110-144	0.9	0.7	1.0	34	40.1	31.5
Cell #2	1-9*	4.7	—	(18.4)	40.6	—	(10.4)
(L7)	5-39	3.0	—	(9.6)	40.6	—	(12.8)
225 μ V	40-139	1.8	2.0	(3.1)	40.6	37	(23.2)
	140-219	1.2	1.6	(2.6)	40.6	32.7	(19.4)
Cell #3	5-39	4.0	—	(29.4)	46.9	—	(6.1)
(L7)	40-119	2.2	3.2	(16.1)	46.9	30.2	(6.0)
437 μ V	120-221	1.4	1.5	(2.6)	46.9	41.6	(23.6)
Cell #4	5-100	0.9	0.7	0.8	27	34.1	29.9
(L7)	101-200	0.6	0.5	0.8	27	31.9	21.0
108 μ V	201-218	0.4	0.3	0.5	27	35.7	22.7
Cell #5	2-39	3.5	—	10.5	16.8	—	5.6
(LBS-3)	40-79	2.4	1.9	3.4	16.8	21.1	11.5
126 μ V							
Cell #6	20-89	2.2	2.2	2.7	13.9	14.0	11.2
(L16)	90-159	1.8	1.5	1.6	13.9	16.9	15.3
178 μ V	160-229	1.7	1.5	1.8	13.9	15.3	13.4
I.S.I. = 30 sec							
Cell #4	5-100	1.2	1.1	1.7	27	27.6	18.6
(L7)	101-136	1.0	0.9	1.8	27	27.3	14.7
68 μ V							
Cell #5	5-69	2.2	3.1	8.0	19.6	13.6	5.2
(LBS-3)	70-139	2.0	2.2	3.2	19.6	18.3	12.2
95 μ V	140-213	2.0	2.1	4.2	19.6	18.5	9.2
I.S.I. = 60 sec							
Cell #2	1-19*	2.9	—	14.7	31.3	—	6
(L7)	20-119	2.1	2.2	2.9	31.3	29.4	22
94 μ V	120-204	1.2	1.2	1.5	31.3	32.1	26.3
Cell #3	5-29	2.7	—	8.3	43.7	—	14.1
(L7)	30-89	1.8	2.3	2.5	43.7	35.8	32
212 μ V	90-205	0.8	0.8	1.1	43.7	44.9	31.3
I.S.I. = 1 sec							
Cell #4	5-100	0.9	0.8	1.5	27	29	17.2
(L7)	101-200	0.7	0.6	0.8	27	33.8	23.1
40 μ V	201-258	0.5	0.4	0.8	27	32.6	17.0

() Corrected for noise.

* Not included in histograms and calculation.

produce behavior habituation or synaptic depression. We therefore stimulated the sensory neurons intracellularly for 200 consecutive stimuli, at frequencies that produced habituation (10- to 60-sec intervals). We then divided each run into two to three consecutive stable "plateau regions" (in which the average EPSP did not decrease by more than 15%) containing 30 to 100 EPSPs, using the means of consecutive groups of 10 successive EPSPs as the index of stability. We then calculated and compared m and $\bar{q}$ values in the consecutive plateau regions and used them as an index of the synaptic depression accompanying repeated stimulation. At this reduced level of transmitter release the EPSPs were 1-5% of the comparable EPSPs at normal release. Thus, the 200 consecu-

tive stimuli are roughly comparable to 15 stimuli of a behavioral habituation training session and each consecutive region at low release is comparable to consecutive groups of five EPSPs at high release.

We estimated m (and $\bar{q}$) in three ways.

(1) *Frequency histograms.* Amplitude histograms were made of the different plateau regions. The first peak was the failures and the next peak was assumed to represent the quantal unit (EPSP) size $\bar{q}_1$. We then calculated m_1 from the equation $m_1 = \bar{E}/\bar{q}_1$ where $\bar{E}$ is the mean amplitude of all the EPSPs in that plateau region. In 11 experiments (Table 1), the estimated m_1 ranged from 4.0 to 0.4 and those of quantal size ranged from 47 μ V to 14 μ V. These values for $\bar{q}$ are similar to those obtained by Miledi (11) at the squid giant synapse. As might be expected from quantal release, the amplitude histograms were multimodal and successive peaks roughly corresponded to integral multiples of the unit peak (Fig. 3A-D). With repetition (compare regions 1, 2, 3 in Fig. 3A-D) the position of the peaks including the unit peak did not change but their amplitude was altered. The incidence of failures increased progressively and the mode of the evoked EPSPs shifted from the 3 to 4 quantal unit peaks (in region 1) to the unit and failure peaks (in region 3). This finding suggests that the EPSP depression during continued stimulation is due to a decrease in quantal content (m) while quantal size ($\bar{q}_1$) remains relatively unaffected. Similar changes occurred at all intervals (1, 10, 30, 60 sec) examined (Table 1).

(2) *Failure analysis.* One can obtain a second estimate of m (m_2) from the Poisson equation by examining the ratio of failures (n_0) to total number of trials (N) where $m_2 = 1n/N/n_0$. These estimates of m_2 (range 3.2-0.3) and $\bar{q}_2$ (range 50 to 14 μ V) based on failures were highly correlated* with those obtained from the amplitude histograms (Table 1). Again, with continued stimulation m decreased while $\bar{q}_2$ remained constant (Table 1).

To determine the fit between the observed data and a Poisson distribution, we used m_1 and the Poisson equation [assuming a 30% coefficient of variation (10) in the unit response] to predict the number of responses containing 0, 1, 2, etc. quantal units. We found good agreement between the predicted and observed values (Figs. 3A-D)^f. We also checked whether the failures tended to occur in groups, indicating possible branch block, using the Poisson equation e^{-2m} ; e^{-3m} , etc. to predict the occurrence of doublets, triplets, etc. In no case did we see an excess of successive failures beyond the frequencies predicted by the Poisson equation.

(3) *Coefficient of variation.* In a Poisson distribution the coefficient of variation is equal to $1/\sqrt{m}$. To arrive at a third estimate of m we used $m_3 = \bar{E}^2/\sigma^2_E$ where σ^2_E is the variance of the EPSPs' amplitudes and $\bar{E}$ is their mean amplitude. Because of the low signal-to-noise ratio, a correction for the noise was sometimes used: $m_3 = \bar{E}^2/\sigma^2_E - \sigma^2_n$ where σ^2_n is the variance of the noise obtained by measuring the baseline at a fixed interval (2 sec) before the occurrence of the spike. This technique is the least reliable of the three and tends to under-

* Pearson correlation test (r), $r = 0.8$, $p < 0.01$ ($N = 26$).

^f A χ^2 test was performed between predicted and observed values of the successive peaks (degrees of freedom = 1 less than the total of groups having a minimum predicted frequency of 5); in 17 of 18 cases the expected and observed values were not statistically different ($p > 0.05$). The values occurring between the peaks were distributed in proportion to adjacent peaks.

estimate $\bar{q}$ (23)*. The estimate of m was consistently higher and $\bar{q}_3$ consistently lower than with the other two estimates (Table 1). Nonetheless, the trend was the same: with repeated stimulation m decreased while $\bar{q}_3$ remained the same.

Quantal Estimates of Depression at Normal Levels of Release.

In two preliminary experiments (in two preparations) we estimated m_3 and $\bar{q}_3$ at normal levels of release using the coefficient of variation and assuming a Poisson process. The results were also consistent with an unvarying $\bar{q}$ value (average 17 μ V) and an average decrease in m (from 85 to 55).

DISCUSSION

A quantal analysis suggests that the critical change accompanying habituation of the gill-withdrawal reflex is a decrease in the number of transmitter quanta released by the presynaptic spike in the sensory neurons. With repeated stimulation the value of $\bar{q}$ remains constant while the value of m decreases by more than 50%. Thus, in six cells examined at 10-sec intervals the mean normalized value for q_2 (or q_1) in the three consecutive plateau regions was 100%, 104%, and 98%, respectively, while that for m_2 (or m_1) decreased from 100% to 64% to 46%. Similar trends were found at 30- and 60-sec intervals (Table 1). This suggests that with repeated stimulation the sensitivity of the postsynaptic receptor is unaffected while the number of quantal units being released is decreased.

There is considerable uncertainty in the application of the coefficient of variation method to small signals found in central neurons. But the graphic method of quantal analysis (combined with averaging) and the analysis of failure based on the Poisson equation gave internally consistent results, and support the finding of Miledi (11) that, despite small quantal size, the quantal analysis can be applied successfully to the central neurons of invertebrates. Because invertebrates are proving highly advantageous for studying behavior, the use of quantal analysis opens the possibility of studying the mechanism of synaptic changes that underlie other behavioral modifications and of analyzing their interrelationships. Thus, a critical question in the study of memory is whether short and long-term memory represent two distinct processes or different stages in a single process. A determination of whether quantal size also remains constant during the synaptic depression accompanying long-term habituation could provide one answer to this question. Similarly, a quantal analysis of the synaptic facilitation accompanying dishabituation (7) could provide further insight into its relationship to habituation.

* The coefficient of variation estimates are less reliable than the failure technique when the value of p is larger or n is lower than demanded by the Poisson assumptions. Also, the estimates of m are unduly sensitive to variations in the synchronization of release and will be higher than the estimates derived from the failure method when release is slightly desynchronized (23).

The finding that the mechanism underlying short-term habituation is presynaptic makes it interesting to analyze morphologically the changes in the number and distribution of the synaptic vesicles (the likely storage sites of transmitter quanta) in the terminals of the sensory neurons. Techniques for marking the presynaptic terminals of identified neurons for electronmicroscopic examination have recently been developed (24) and could permit a subcellular analysis of this and related instances of behaviorally relevant synaptic plasticity.

We have benefitted from discussions with Drs. G. Fishbach and J. Koester and from their comments and those of T. J. Carew on an earlier draft of this manuscript. We also thank Kathrin Hiltner for help with the illustrations. This research was supported by Research Career Development Award 5K04 NS 70346-02 to V.C., Research Scientist Award MH 18,558 to E.R.K. and NIH Grants MHS-19795 and NS-09361.

1. Thorpe, W. H. (1958) in *Learning and Instinct in Animals* (Harvard University Press, Cambridge), pp. 49-67.
2. Hinde, R. A. (1970) in *Animal Behaviour* (McGraw-Hill, New York), 2nd ed., p. 876.
3. Thompson, R. F. & Spencer, W. A. (1966) *Psychol. Rev.* **73**, 16-43.
4. Jeffrey, W. E. (1968) *Psychol. Rev.* **75**, 323-334.
5. Cohen, L. B. (1973) *Merrill-Palmer Quart. Behav. Develop.* **19**, 157-180.
6. Carew, T. J. & Kandel, E. R. (1974) in *Synaptic Transmission and Neuronal Interaction*, ed. Bennett, M. V. L. (Raven Press, New York), pp. 339-383.
7. Castellucci, V., Pinsker, H., Kupfermann, I. & Kandel, E. R. (1970) *Science* **167**, 1745-1748.
8. Zucker, R. S. (1972) *J. Neurophysiol.* **35**, 621-637.
9. Spencer, W. A., Thompson, R. F. & Neilson, D. R. (1966) *J. Neurophysiol.* **29**, 253-274.
10. Kuno, M. (1971) *Physiol. Rev.* **51**, 647-678.
11. Miledi, R. (1967) *J. Physiol.* **192**, 379-406.
12. del Castillo, J. & Katz, B. (1954) *J. Physiol.* **124**, 560-573.
13. Byrne, J., Castellucci, V. & Kandel, E. R. (1974) *J. Neurophysiol.* **37**, 1041-1064.
14. Frazier, F. W. T., Kandel, E. R., Kupfermann, I., Waziri, R. & Coggeshall, R. E. (1967) *J. Neurophysiol.* **30**, 1288-1351.
15. Kupfermann, I., Carew, T. J. & Kandel, E. R. (1974) *J. Neurophysiol.* **37**, 996-1019.
16. Pinsker, H., Kupfermann, I., Castellucci, V. & Kandel, E. R. (1970) *Science* **167**, 1740-1742.
17. Kupfermann, I., Castellucci, V., Pinsker, H. & Kandel, E. R. (1970) *Science* **167**, 1743-1745.
18. Kandel, E. R. & Taub, L. (1965) *J. Physiol.* **181**, 28-47.
19. Kandel, E. R., Frazier, W. T., Waziri, R. & Coggeshall, R. E. (1967) *J. Neurophysiol.* **30**, 1352-1376.
20. Kehoe, J. (1972) *J. Physiol.* **225**, 147-172.
21. Frankenheuser, B. & Hodgkin, A. L. (1957) *J. Physiol.* **137**, 218-244.
22. Austin, G., Yai, H. & Sato, M. (1967) in *Invertebrate Nervous System*, ed. Wiersma, C. A. G. (University of Chicago Press), pp. 39-53.
23. Williams, J. D. & Bowen, J. M. (1974) *J. Theor. Biol.* **43**, 151-165.
24. Thompson, E. B., Schwartz, J. H. & Kandel, E. R. (1973) *Neurosci. Soc. Abstr.* **3**, no. 47.3.