stimulus, such as might be caused by the beginnings of an undertow, is a reliable predictor that *Aplysia* is about to be torn from its footing and smashed against a rock by an incoming wave, it will clearly benefit by recognizing this contingency and tucking its more delicate organs safely out of the way.

Other members of the species live deeper in the sea, in the sublittoral zone, where they are rarely exposed to air. These creatures can afford to be more lackadaisical, as life is a lot calmer in the sublittoral zone.

2.1.3 How Does *Aplysia* Learn?

Aplysia's nervous system contains about 20,000 central neurons [28]. These are grouped into nine ganglia, each of which contains about 2000 neurons. An important and well-studied part of the circuitry for the gill withdrawal reflex resides in its abdominal ganglion. The complete circuit is comprised of some fifty or so neurons, including a large number of interneurons [19]. The circuit can be reduced to a simplified form containing only the primary monosynaptic connections and a few interneurons; the result is shown in Figure 2-4. The basic reflex is implemented by direct connections from sensory neurons that respond to tactile stimulation of the siphon and motor neurons that contract the gill.

Sensory neurons from the tail also have direct reflex connections to the motor neurons, but in addition to the direct connections they connect via facilitatory interneurons to the presynaptic terminals of the siphon sensory neurons. In the following discussion, we will focus on what goes on inside these presynaptic terminals, as this is the primary locus for the known mechanisms underlying classical conditioning in *Aplysia*.[2]

[2]Readers who are unfamiliar with neuroscience may wish to refer to Appendix A for background that will be helpful in following the material in this section.

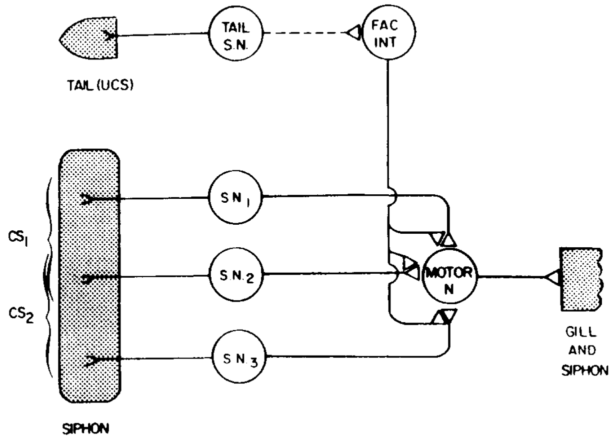

Figure 2-4: A diagram of the simplified Aplysia neural circuit. Sensory neurons from the siphon make direct contact on the motor neurons controlling the gill withdrawal reflex. Tail sensory neurons act via facilitator interneurons, which connect to the reflex pathway through synaptic contacts on the presynaptic terminals of synapses between the sensory and motor neurons. The tail sensory neurons also make direct contacts with motor neurons (not shown). (From [21].)

Habituation

In habituation, repeated firing of a sensory neuron reduces the responsiveness of the synapse. The hypothesized mechanism by which this occurs is the inactivation of a percentage of the voltage-sensitive calcium channels in the presynaptic terminal [28].[3] These channels are embedded in the cell membrane, and they normally open in response to the depolarization which occurs during an action potential. This allows calcium ions (Ca^{++}) to diffuse into the cell from the extracellular fluid. Ca^{++} then binds to vesicles of neurotransmitter and catalyzes their release into the synaptic cleft [27].[4]

When these channels are inactivated during habituation, it reduces the rate at which Ca^{++} can diffuse into the synapse, which in turn reduces the amount of trans-

[3] Although these experiments are relatively old, more recent work has concentrated on associative and/or long-term learning, so this explanation for the habituation response is still current.

[4] See Appendix B.4 for information about cellular actions of Ca^{++}.

mitter released in response to an action potential. Calcium channel inactivation accounts only for short-term habituation, as the channels decay back to their normal resting state within a few hours.[5]

The mechanism for long-term habituation is not known.

Sensitization

Sensitization was found to act via a much more complex mechanism that is initiated by transmitter release from facilitator interneurons [30].

Serotonin, the putative transmitter released by the facilitator interneuron, acts on the presynaptic terminal via a metabotropic receptor.[6] Unlike ionotropic receptors, which act through electrical conductance changes, the serotonin receptor is coupled to a G protein which stimulates adenylate cyclase activity. Adenylate cyclase synthesizes cyclic AMP, a common cellular second messenger which controls a wide range of metabolic actions in different cell types [51]. It has been hypothesized that cyclic AMP acts exclusively through a cyclic AMP-dependent protein kinase, which phosphorylates a specific set of substrate proteins when activated by cyclic AMP. Phosphorylation changes the activity of the substrate protein, in a way that is dependent on the particular protein and phosphorylation site.[7]

In the case of sensitization in *Aplysia*, the cyclic AMP-dependent protein kinase apparently affects two potassium (K^+) channels that normally open to repolarize

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[5]Ion channels can take on a number of distinct conformational states [47]. The probability of a state transition is determined by the relative energy and stability of the two states. This Ca^{++} channel, for example, has three identifiable states: open, closed, and inactivated. The closed state has the lowest energy under equilibrium conditions, and is therefore the channel's resting state. The state transition from closed to open is most probable when the membrane is depolarized, which changes the relative energy of the two states.

State transitions from the open to the inactive state appear to be common, whereas transitions from closed to inactive are rare. This hypothesis corresponds to the observation that repeated depolarization results in a population shift toward the inactive state (that is, given a population of Ca^{++} channels, there is an increase in the percentage of channels that are inactive.) The inactive state appears to be highly stable, for once a Ca^{++} channel is inactivated, it is likely to remain inactive for an extended period.

[6]See Appendix B for background information on neurotransmitter receptors and their actions.

[7]Appendix C contains a brief introduction to protein phosphorylation.

the presynaptic terminal at the end of an action potential [22]. These channels are inactivated by phosphorylation, which slows repolarization and thereby prolongs the action potential.

The duration of the action potential affects transmitter release via the same voltage-sensitive Ca^{++} channels that mediate short-term habituation. The Ca^{++} channels remain open as long as the terminal is depolarized, so the amount of Ca^{++} that flows into the cell is proportional to action potential duration. The lengthening of the action potential caused by K^{+}channel inactivation therefore increases Ca^{++} influx, which in turn increases transmitter release.

In addition to increasing transmitter release, serotonin also increases the excitability of sensory neurons. This effect may be due to a class of serotonin receptor that is distinct from that involved in spike broadening [45].

Again, these mechanisms explain short-term but not long-term sensitization. The increase in action potential duration lasts only as long as the increase in intracellular cyclic AMP [28]. The cyclic AMP concentration remains elevated for over an hour after a single brief pulse of serotonin, however, which is in sharp contrast to the millisecond time scale of the electrical effects of ionotropic receptors.

Associative Learning

The hypothesized mechanism for associative learning [1] makes use of the same facilitator interneurons that are involved in sensitization.

Strong stimulation of the sensory neurons in the tail (the US) activates the gill withdrawal motor neurons directly (this is the unconditioned response, or UR), and it also activates the facilitator interneurons. This sensitizes the sensory synapses, increasing the likelihood that any tactile stimulus will elicit a withdrawal response. However, if another sensory neuron (the CS) fires shortly before the facilitator interneuron, the amplification of the withdrawal reflex for that stimulus is greatly enhanced, with an effect that may last several days.

The learned association between the US and the CS may occur as a result of a second function of Ca^{++}. Once it enters a cell, Ca^{++} is very quickly bound to calmodulin. Like cyclic AMP, the Ca^{++}-calmodulin complex is a common cellular second messenger. One of the observed actions of Ca^{++}-calmodulin is increased activation of adenylate cyclase [1, 8].

The hypothesized effect of Ca^{++}-calmodulin on adenylate cyclase in the *Aplysia* synapse is modulatory. It does not directly activate cyclic AMP production, but it enhances the effectiveness of serotonin activation. When Ca^{++}-calmodulin is active, therefore, a greater amount of cyclic AMP will be produced in response to the same amount of serotonin.

The strength of associative learning depends strongly on the ISI, that is, the time delay between the CS and the US. The associative effect is transient, peaking and decaying within two seconds in the aftermath of an action potential [1]. Only if the facilitator interneuron fires within this time window is the sensitization effect enhanced, resulting in a differential response at that synapse.

In order for these mechanisms to produce associative learning, the US and the CS must each be capable of producing a withdrawal response alone, and there must be a facilitator interneuron connecting the US with the output terminal from the CS. The need for these pre-existing connections limits the organism's learning ability. Only stimulus-response pairings which are already in its behavioral repertoire can be learned. There are a huge number of facilitator interneurons in *Aplysia* [28], which is what we would expect if this hypothesis is correct: the more abundant these neural connections, the more versatile the animal will be in responding to its environment.

Another interesting property of these mechanisms is the parsimony of neural connections: the same connections that are necessary for sensitization, a general arousal mechanism, are also used to produce a selectively enhanced response to stimuli that consistently precede the US.

The mechanisms for these three forms of learning (habituation, sensitization, and

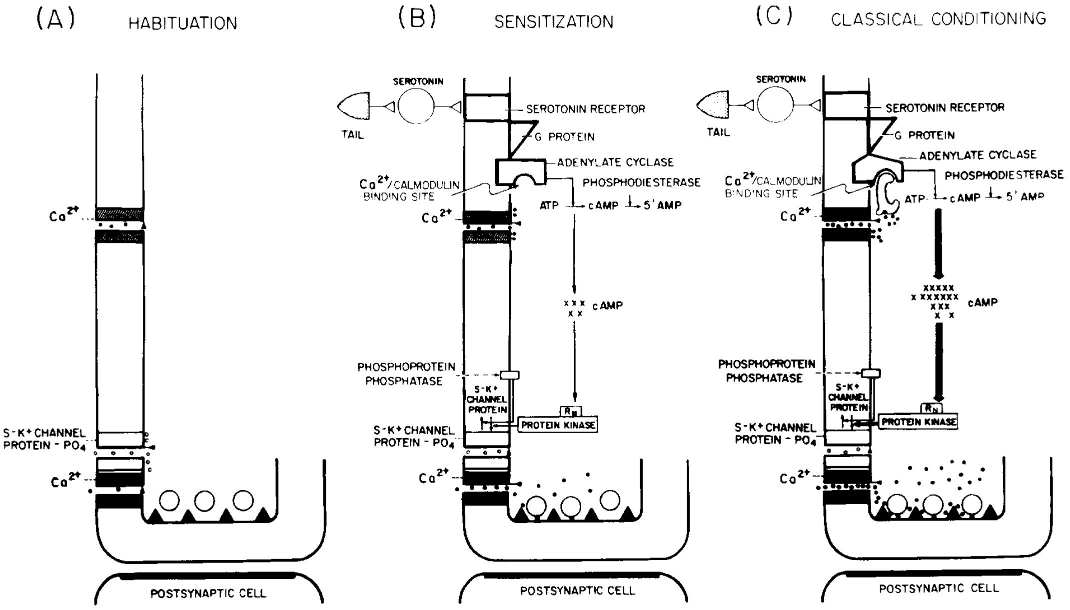

Figure 2-5: Summary of the mechanisms hypothesized to mediate (a) habituation, (b) sensitization, and (c) associative learning in Aplysia's gill withdrawal reflex. Each image shows the interior of a presynaptic terminal, and diagrams the chemical processes described in the text. (From [20, page 76].)

associative learning) are summarized in Figure 2-5.

Higher Order Learning Phenomena

Hawkins and Kandel, in response to these findings, speculate on the existence of a "cell-biological alphabet for learning" [21]. That is, they hypothesize the existence of a small set of primitive cellular mechanisms that can be combined to produce a wide range of learning phenomena. They describe how mechanisms already found could, in principle, be combined to produce other learning phenomena that have not yet been studied.

- Extinction and recovery were hypothesized to work via the same mechanism that mediates habituation.

- Second order conditioning is explained by introducing additional neural connections that cause a CS sensory neuron to excite the facilitator interneuron (IN). Synapses from the CS to the IN are also facilitated (their behavior is hypothesized to match a Hebbian learning model, unlike the CS-CR synapses). Thus, the ability of the CS to excite the IN is enhanced as it is conditioned. Once conditioning is complete, the CS can facilitate other stimuli through the IN in the same way the US did originally.

- Generalization across similar tactile stimuli was explained by the existence of overlapping receptive fields for many sensory stimuli, so that a similar stimulus would excite a subset of the sensory neurons that were conditioned.

- Additional properties of the facilitator IN were introduced to explain blocking. The IN fires only at the onset of a sustained input, so if a fully conditioned CS_1 fires before the US, it can block the facilitator from firing in response to the US neuron. This will in turn prevent facilitation of the new CS_2. Because there is no time delay between firing of CS_1 and CS_2, the response of the IN to CS_1

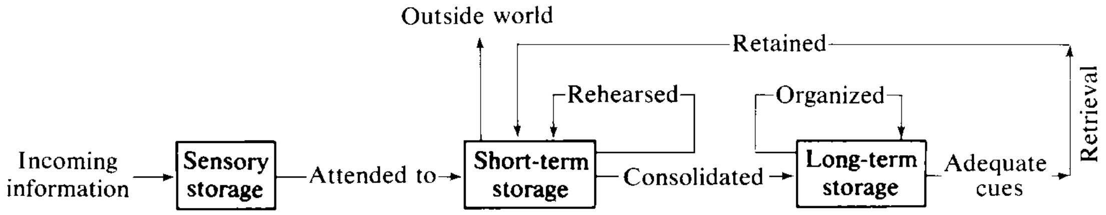

Figure 2-6: Diagram showing hypothetical relationship between short-term and long-term forms of learning (From [27, page 619].)

does not produce conditioning. If a response in only partly conditioned, the facilitator will be only partly blocked, and some conditioning of CS_2 will occur.

- Pre-exposure to the US may cause conditioning to the background environment, which in turn would block conditioning to a CS which is presented later.

Although these learning phenomena have not actually been observed in *Aplysia*, they are found in Limax maximus, a close relative [16, 57].

Mechanisms for Long-Term Learning

Following the training trials, as the short-term response decays, there is a progressive shift toward longer-term forms of learning. This shift occurs gradually over the course of several hours. Figure 2-6 shows a diagram of the hypothetical relationship between these two forms of learning.

Mechanisms for long-term learning have not yet been extensively explored, though it appears to involve both the production of new proteins [12, 65][8] and new synapse formation [17].

One apparent mechanism for DNA transcription in long-term sensitization is

[8]There are two major processes which can be blocked to test for the involvement of protein production in a cellular process. The first is DNA transcription, which produces new strands of messenger RNA (mRNA). The second is translation of mRNA into amino acid sequences during protein synthesis.

cyclic AMP-dependent phosphorylation of a substrate protein that acts as a DNA transcription factor [12]. This protein, when activated by cyclic AMP, binds to a particular DNA sequence and activates (or, conversely, inhibits) transcription of the associated genes.

What, then, is the function of the proteins encoded by these up- or down-regulated genes? One answer is that they may lead to long-term changes in phosphorylation of the same set of substrate proteins that is phosphorylated in a short-term manner by the presence of cyclic AMP in the presynaptic terminal [65]. In this case, long-term facilitation would be mediated by long-term spike broadening, via the long-term inactivation of K^+channels.

One set of proteins which are down-regulated during long-term sensitization has been identified as an NCAM-related class of cell adhesion molecules, which attach to the external surface of sensory neurons [29]. NCAMs are important in regulating cell growth during development, and may be part of the mechanism for new synapse formation.

2.2 A Simulation of the *Aplysia* Circuit

This section describes a simulation of the *Aplysia* gill withdrawal circuit. Individual synapses are the primary site of learning in this network, so the model concentrates on the electrical and chemical mechanisms within a single synapse.

There are at least six distinct learning mechanisms in the circuit, which result in short and long term forms of habituation, sensitization, and associative learning, and which allow for the possibility of higher-order forms of learning such as differential conditioning and blocking. Only the short-term forms of learning are modelled here.

Some of these mechanisms have previously been modelled elsewhere. Byrne and Gingrich [8] model the effects of chemical diffusion and synaptic vesicle transport within the synapse, which captures the effect of synaptic depression due to vesicle depletion. The model presented by Hawkins [20] demonstrates his hypotheses about higher-order learning phenomena, as described in [21].

The computational model presented here is a straightforward instantiation of the conceptual models used in biology, and a major purpose of the simulation is to test the adequacy of current conceptual models for short-term learning. For this reason, certain aspects of the experimental data are considered more rigorously than in other models. Parameter selection is based directly on experimental data, and tested against experiments which use different protocols, rather than selected to maximize the desired result [20] or left unspecified [8]. The model derived here for the dependency of transmitter release on Ca^{++} influx explains aspects of the experimental data that cannot be explained using the simpler models used in [8] and [20]. The process underlying associative learning is also modelled in a novel manner that better predicts the observed effect of the ISI on the strength of learning.

2.2.1 Simulation Equations

Individual action potentials are treated as atomic events which can occur at 10 msec intervals.[9] However, state variables, such as concentrations of chemicals in the presynaptic terminal, are updated only when their values are needed to model the cell's response to new events. The short-term dynamics of individual learning trials can be modelled in this way without the need to step the system through large numbers of small time steps during extended periods (e.g. between learning trials) when nothing interesting is going on.

Four state variables are used to model the environment inside the presynaptic terminal of a sensory neuron. These variables track the factors hypothesized to mediate conditioning in the synapse. A diagram of the simulated system is shown in Figure 2-7.

[9] The duration of an individual action potential is on the order of 3-4 msec, which is much shorter than this 10 msec time scale.

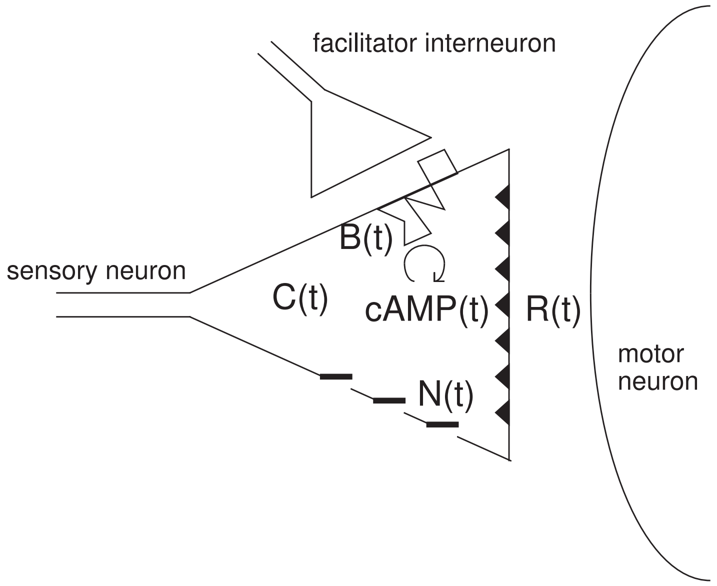

Figure 2-7: A diagram showing the state variables used in the simulation model. [N](t) is the concentration of active calcium channels; [cAMP](t) is the cyclic AMP concentration; [C](t) is the concentration of Ca^{++}-calmodulin, and [B](t) is the concentration of Ca^{++}-calmodulin bonds with adenylate cyclase. [R](t) represents transmitter released into the synaptic cleft.

When a Ca^{++} channel is inactivated during habituation, it ceases to be available for influx of calcium during an action potential. [N](t), the concentration of available Ca^{++} channels, is used to track this effect.

The sensitization effect involves a cascade of several chemical events, but it is rate-limited by the concentration of cyclic AMP [30].[10] It is modelled here with a single variable, [cAMP](t), representing the cyclic AMP concentration. The dynamics of protein kinase activation, protein phosphorylation, and K^{+}channel inactivation are assumed to follow cyclic AMP changes closely, and are not modelled separately.

The factors mediating associative learning are more complex. Again a chemical cascade is involved, but here the dynamics of the interaction between Ca^{++}, calmodulin, and adenylate cyclase are modelled to capture the effect of the ISI on associative learning. Two state variables are used, [C](t) and [B](t). [C](t) is the concentration of free Ca^{++}-calmodulin, and [B](t) is the concentration of Ca^{++}-calmodulin bound to adenylate cyclase.

Behavior in the Absence of Activity

When there is no activity in the sensory neuron or the facilitator, state variables decay to their equilibrium values according to the following equations:[11]

$$[\mathrm{N}](t) = N_0 - (N_0 - [\mathrm{N}](t_0)) \cdot e^{(t-t_0)/\tau_N} \tag{2.1}$$

$$[\mathrm{cAMP}](t) = [\mathrm{cAMP}](t_0) \cdot e^{(t-t_0)/\tau_{cAMP}} \tag{2.2}$$

$$[\mathrm{C}](t) = c_1 e^{m_1(t-t_0)} + c_2 e^{m_2(t-t_0)} \tag{2.3}$$

[10] That is, the change in action potential duration appears to be primarily controlled by an ongoing elevation of the cyclic AMP concentration, rather than by some other step in the chemical cascade.

[11] These equations are derived from standard rate equations for chemical reactions (see [25] for an introduction to this topic.) Linear kinetics are assumed for simplicity, except where otherwise indicated.

$$[\mathrm{B}](t) = c_1 a_1 e^{m_1(t-t_0)} + c_2 a_2 e^{m_2(t-t_0)} \tag{2.4}$$

where t_0 is the time of the most recent event, $[\mathrm{N}]_{\max}$ is the total concentration of Ca^{++} channels, τ_N is the time constant for Ca^{++} channel recovery, τ_{cAMP} is the time constant for the cyclic AMP concentration decay, and m_1, m_2, c_1, c_2, a_1, and a_2 are determined by the reaction rates between Ca^{++}-calmodulin and adenylate cyclase and the initial conditions at time t_0 (see Section 2.2.4 for a derivation of these parameters.)

Response to Events

There are two kinds of events that cause step changes in the internal state of the presynaptic terminal. The first is an incoming action potential from the sensory neuron; the second is an action potential in the facilitator interneuron.

Sensory Action Potentials The concentration of cyclic AMP is used to determine the duration of an action potential in the sensory neuron.

$$\mathrm{D}(t) = D_0(1 + K_D \cdot [\mathrm{cAMP}](t)) \tag{2.5}$$

where D_0 is the initial duration in the absence of any learning effects, and K_D is the magnitude of cyclic AMP's effect on action potential duration.

The action potential has a primary effect on the rate of Ca^{++} influx ($[\mathrm{I_{Ca}}](t)$), which determines both the amount of transmitter released ([R]) and the concentration of Ca^{++}-calmodulin. It also changes the concentration of inactivated Ca^{++} channels, which changes the rate of Ca^{++} influx for future action potentials. The effect of an action potential is modelled as follows:

$$[\mathrm{I_{Ca}}](t) = I_{Ca0}[\mathrm{N}](t) \tag{2.6}$$

$$\Delta[\mathrm{C}](t) = [\mathrm{I_{Ca}}](t) \cdot \mathrm{D}(t) \tag{2.7}$$

$$\Delta[\mathrm{N}](t) = -[\mathrm{N}](t)(1 - K_N) \quad (2.8)$$

$$[\mathrm{R}](t) = R_0 \left(\frac{[\mathrm{I_{Ca}}](t)}{I_{Ca0}}\right)^4 \left(\frac{\mathrm{D}(t)}{D_0}\right)^5 \quad (2.9)$$

I_{Ca0} is the Ca^{++} influx per Ca^{++} channel per unit time, R_0 is the transmitter released by a single sensory action potential in the absence of learning, and K_N is the percentage of Ca^{++} channels still active after an action potential.

The magnitude of the gill withdrawal response is assumed to be proportional to transmitter release.[12] Equation 2.9 is derived in Section 2.2.4.

Facilitator Action Potentials The other type of event is an action potential in the facilitator interneuron. The primary effect of this is a change in the cyclic AMP level, which is modelled as instantaneous.[13]

$$\Delta[\mathrm{cAMP}](t) = cAMP_0(1 + K_{cAMP} \cdot [\mathrm{B}](t)) \quad (2.10)$$

$cAMP_0$ is the cyclic AMP released per facilitator action potential in the absence of Ca^{++}-calmodulin, and K_{cAMP} is the percentage increase in cyclic AMP production in response to a unit concentration of Ca^{++}-calmodulin bonds with adenylate cyclase.

Parameter Selection

The parameters in the model correspond to specific physical quantities whose value can be determined from the experimental data.

[12] In biological experiments, this response has been measured in terms of the magnitude of the excitatory post-synaptic potential (EPSP) in the motor neuron, the number of motor neuron spikes generated by the EPSP, the magnitude of the withdrawal response (that is, how far the gill is retracted), or the duration of the withdrawal response. The relationship between number of motor neuron spikes and the magnitude of the withdrawal response is approximately linear [27, page 551].

[13] Again, this is a simplification, since the dynamics of the chemical cascade resulting from the G protein activation of adenylate cyclase must take some time; the assumption here is that the cyclic AMP concentration will have reached its final value before the next event which depends on it. The discrepancy between the behavior of this model and sensitization Experiment 2 (described below) can be explained if the experimental protocol used in that experiment violates this assumption.

For simplicity in interpreting the results, the parameters describing the initial state of the synapse (in the absence of learning) were normalized, so that all results are shown in proportion to the initial state.

$$N_0 = D_0 = I_{Ca0} = R_0 = cAMP_0 = 1.0$$

The "weak tactile stimuli" used in intact animals are assumed to consist of a single action potential in the sensory neuron, in accordance with the response described in [28] (one to two spikes per sensory neuron in response to a tactile stimulus).

Parameters for the calcium channels are set to match the experimental results of habituation Experiments 1 and 2 (described in Section 2.2.2 below.) The habituation coefficient K_N is set to produce habituation to 30% of the initial response after 15 trials at an inter-trial interval of 1.5 minutes, and the recovery rate is set to give 75% recovery 10 minutes later.

$$\begin{aligned} K_N &= 0.896 \\ \tau_N &= 350.0 \text{ seconds} \end{aligned}$$

Parameters controlling the sensitization response were set to match the experimental results for the calibration test described in sensitization Experiment 1 (described in Section 2.2.2.) The value for τ_{cAMP} was estimated from the graph shown in Figure 10(a), and K_D was set to produce a maximum response of 450% of the pre-test level.

$$\begin{aligned} K_D &= 0.0045 \\ \tau_{cAMP} &= 1000.0 \text{ seconds} \end{aligned}$$

There were a number of sensitization and associative learning experiments where the strength of the US was not specified. The value used in the simulation was chosen to match the strength of the initial response in sensitization Experiment 3. The tail shock (and every other noxious stimulus used in experiments other than the calibration test) is assumed to consist of 30 facilitator action potentials at 30 Hz.[14]

The parameter K_{cAMP}, which controls the strength of the associative response, is set using associative learning Experiment 1 (described in Section 2.2.2.) The serotonin puff, like other noxious stimuli, was assumed to produce the equivalent of 30 spikes over its 1 second duration.

$$K_{cAMP} = 13.5$$

The parameters which determine the effect of the ISI on learning are discussed in Section 2.2.4.

2.2.2 Results

Habituation and Recovery

The habituation response in the model was tested against experimental results which illustrate the character of the response in *Aplysia*. These results are:

1. In [30], light tactile stimulation of the siphon at 1.5 minute intervals caused habituation to 30% of the initial response within 10-15 stimuli.

2. The habituation reflex shows 75% recovery 10 minutes after a single training session [27, page 544] (see Figure 8(a).)

[14] The simulation assumes that the amount of serotonin released by a single facilitator action potential does not change with repeated activity, and that the relationship between the amount of serotonin released, the amount of cyclic AMP produced, and the increase in sensory action potential duration is linear.

3. Stimulating every 30 seconds, the withdrawal reflex is reduced to 20% of the maximum response after 10 stimuli. After a 10 minute rest, the reflex recovers to over 90% of the maximum. The reflex habituates more quickly during rehabituation after resting [27, page 544] (see Figure 9(a).)

4. Habituation to 50% of the maximum response occurs with an intertrial interval of half an hour [30] (see Figure 10(c).)

5. Less habituation is observed when stronger tactile stimuli are used [27, page 545] (see Figure 9(d).)[15]

The recovery time constant τ_N for the habituation tests was calculated using the data from Experiment 2, and the habituation coefficient K_N was then set to match the habituation data for Experiment 1. These parameters were then tested using the experimental protocols for Experiments 3 and 4.[16] Test results are shown in Figures 2-8 and 2-9.

Sensitization

The following experiments, taken from the *Aplysia* literature, were used to evaluate the model's sensitization response.

1. In a fully habituated synapse receiving sensory stimulation at 10 second intervals, a facilitating stimulus was presented at 6 Hz. for 10 seconds. This resulted in facilitation to 450% of the habituated response strength, which decayed over the course of an hour to about 150% of the habituated response. The time course closely matched the decay in cyclic AMP over the first hour following incubation with serotonin [28] (see Figure 10(a).)

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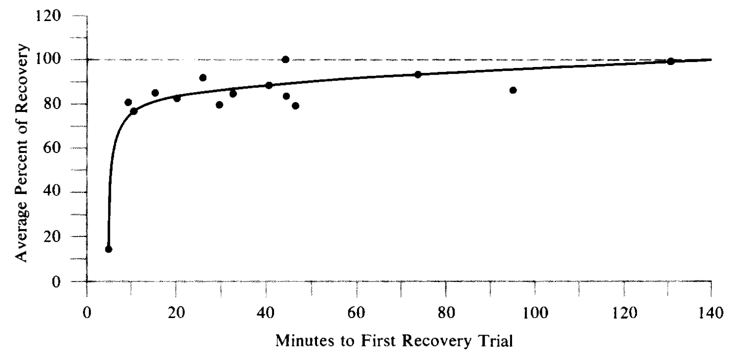
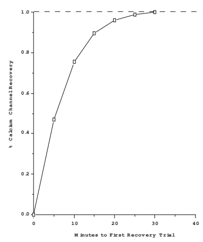
[15] The strength of the tactile stimulus is measured in terms of the force of water emitted from a water jet, which is used to apply the stimulus.

[16] Experiment 5 was not tested, as it was clear from the start that no mechanism is present in the model that can produce this result.

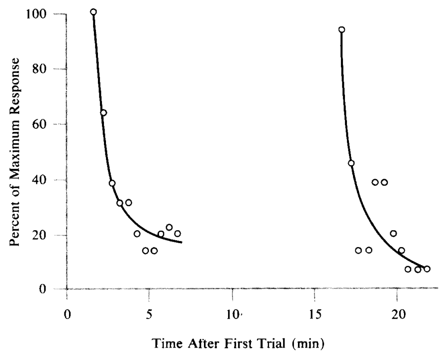


(a)

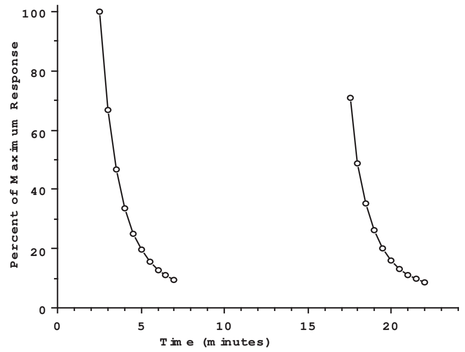


(b)

Figure 2-8: Results of recovery experiment. (a) Percent recovery of Aplysia's habituation reflex, tested at different recovery intervals following a single habituation session. (From [27, page 544].) (b) Recovery in the model using same experimental protocol, with a training session consisting of 15 trials at 1.5 minute intervals.



(a)



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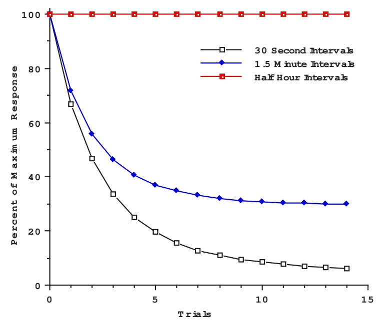

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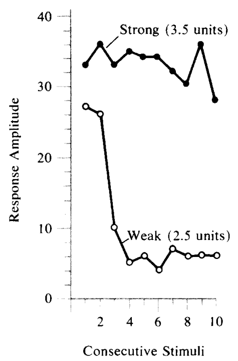


(d)

Figure 2-9: Results of habituation experiments. (a) Habituation, recovery, and rehabituation in Aplysia, using a 30 second intertrial interval and a 10 minute rest after the 10th trial (From [27, page 544].). (b) Habituation in the model using the same experimental protocol. (c) Habituation in the model with three different inter-trial intervals. (d) Degree of habituation found in Aplysia with different stimulus strengths (From [27, page 545].)

2. A sequence of 13 stimuli was presented at 1.5 minute intervals, followed by a strong sensitizing stimulus,[17] followed by another 12 trials [27, page 551]. Sensitization following habituation caused a gradual increase in the response over the first 3 stimuli presented after the sensitizing stimulus before reaching a maximum.

3. Stimulus presentations at half hour intervals following sensitization shows a response that is initially almost double the baseline response. Sensitization decays over the course of four hours to match the level of habituation that would be found if sensitization had not occurred [30].

The sensitization parameters K_D and τ_{cAMP} were determined using Experiment 1, and then tested with the protocols used in Experiments 2 and 3. Test results are shown in Figure 2-10.

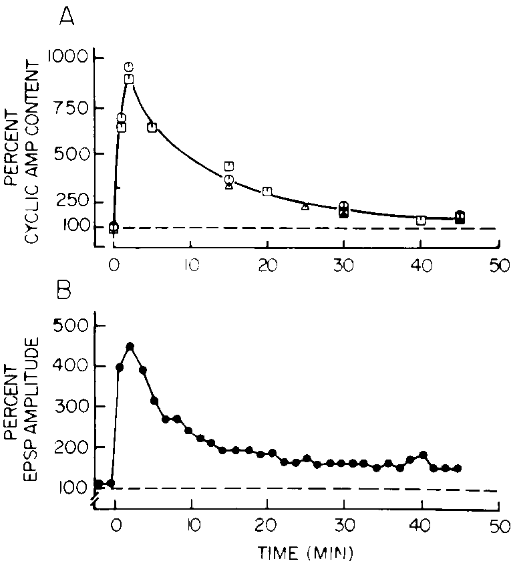
Associative Learning

The following *Aplysia* experiments were used to evaluate associative learning in the model.

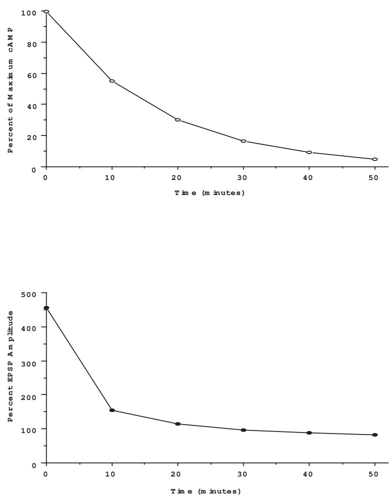
1. A train of 6 spikes at 10 Hz. was immediately followed by a puff of serotonin one second in duration. Cyclic AMP levels were measured at four times the concentration found when an unpaired serotonin puff was used [1, page 228].

2. A weak tactile stimulus in the intact animal was followed immediately by a strong unconditioned stimulus, in 15 training trials at a 5-minute inter-trial interval, followed by a 30 minute rest period. The strength of the withdrawal response was compared to a control group in which the conditioned stimulus was presented 2.5 minutes after the unconditioned stimulus. The strength of the unpaired response was approximately 1.7 times the pre-test response, whereas

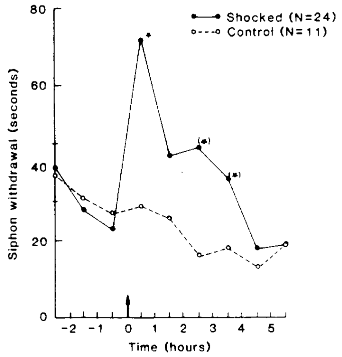
[17] The magnitude of the sensitizing stimulus is not specified.



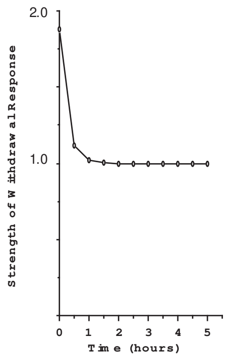
(a)



(b)



(c)



(d)

Figure 2-10: Results of sensitization experiments. (a) Time course of cyclic AMP concentration decay and strength of motor neuron EPSP using 10 second inter-trial intervals (From [28, page 51].) (b) Model behavior using same experimental protocol as in (a). (c) Decay of sensitization of gill withdrawal reflex following the protocol described in Experiment 3 (From [30, page 435].) (d) Model behavior using same experimental protocol as in (c).

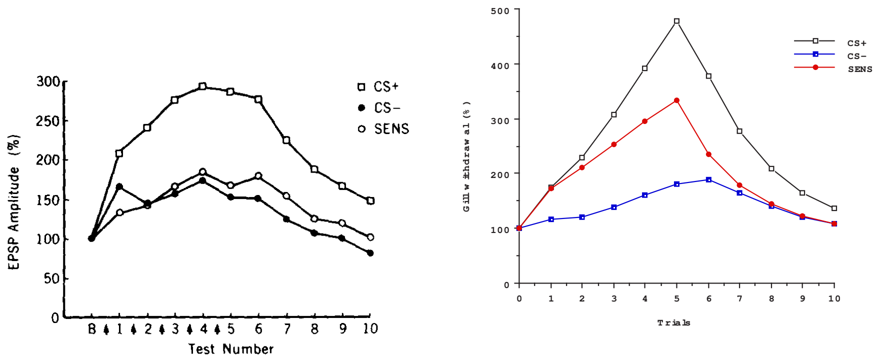
the associative response was over 4 times the pre-test response (measured in terms of duration of gill withdrawal) [1, page 217].

3. In *Aplysia*, with a sequence of 5 training trials at 5 minute intervals, the strength of associative learning stabilized at 300% of the initial response [8]. Response strength was measured at 5 minute intervals, offset from the training trials by 2.5 minutes. Results of this experiment in CS+ (paired stimuli at 600 msec ISI), CS- (CS follows US by 2 minutes), and sensitization only conditions is shown in Figure 11(a).

4. In five training trials at 5 minute intervals, a train of 6 spikes at 10 Hz. was administered to a sensory neuron, immediately followed by a strong tail shock. Again, a control neuron was stimulated 2.5 minutes after the tail shock. An hour after the training trials, the motor neuron EPSP in response to the associated sensory stimulus was nearly double its pre-test amplitude, whereas the response to the control neuron showed no facilitation [1, page 221].

The value of the parameter K_{cAMP} was chosen to match the difference in cyclic AMP levels observed in Experiment 1. With a value of $K_{cAMP} = 13.5$, the cyclic AMP concentrations measured after one sensitization trial and one associative learning trial were 30 and 120, respectively. The model behavior was then tested using the other experiments listed.

The values for the reflex strength obtained using the protocol described in Experiment 2 were 1.4 and 1.8, respectively. Results of Experiment 3 are shown in Figure 11(b). Experiment 4 was not run, because the strength of the associative response after half an hour in Experiment 3 was already well below the value observed after one hour in Experiment 4.



(a) (b)

Figure 2-11: Results of associative learning experiment. (a) Strength of gill withdrawal (as percent of initial strength) in Aplysia in CS+, CS-, and sensitization only test conditions (From [8, page 66].) (b) Model behavior using same experimental protocol.

2.2.3 Discussion

Habituation and Recovery

The conceptual model used by neuroscientists is unable to explain the experimental data on habituation without hypothesizing a more complex set of Ca^{++}channel states.

The time course of habituation and recovery in this model is determined by K_N and τ_N. These parameters were selected so that the model would match the time course of a single habituation experiment well. However, the results of experiments using different inter-trial intervals produce different estimates of the parameter values. For example, the recovery time for the Ca^{++} channels must be very short for recovery to be nearly complete in 10 minutes. However, with a short recovery time, no habituation is seen in the model with a half hour intertrial interval. This inconsistency in the experimental results cannot be resolved without a more complex habituation mechanism. One possible explanation is that long-term habituation may come into play with a half-hour inter-trial interval.

The observed time course of recovery follows a roughly exponential curve, but the tail of the curve is much longer than would be obtained if the recovery rate were constant. This may indicate the presence of a multi-stage recovery process, where the first stage of recovery is much faster than some later stage.

The model is unable to reproduce the experimentally observed enhancement of the habituation response during rehabituation after a recovery period. If Ca^{++} channels are more susceptible to long-term inactivation during the late phase of recovery, this might explain both the presence of habituation with a half-hour inter-trial interval and the enhancement of habituation following recovery. These effects would result if long-term inactivation were to reduce the pool of Ca^{++} channels participating in rapid recovery, which would shift the entire habituation curve downward.

If we are to explain these results with the Ca^{++}channel alone, we would have to hypothesize a more complex channel. A channel which has multiple inactive states with different recovery time constants could explain the observed recovery curve, and could also explain the enhancement of channel inactivation during rehabituation. A multi-stage recovery process, where the channel is more susceptible to long-term inactivation in some later recovery stage, could also explain the data on habituation with a longer inter-trial interval.

The decrease in habituation observed with strong stimuli also cannot be reproduced with this simple habituation mechanism. In fact, the model predicts greater habituation for strong stimuli, not less. The biological effect may be the result of unmodelled connections from the sensory neurons onto the facilitator neuron. When strong stimuli are used, these connections could produce indirect sensitization of the synapse from the sensory to the motor neuron.

Sensitization

The matching time course of decay of sensitization and of the cyclic AMP concentration is cited as evidence for a correspondence between the two. However, because of the assumed nonlinear dependency between action potential duration and strength of the withdrawal response, the time course of the two would not necessarily be expected to match closely.

The observed EPSP shown in Figure 10(a) does not return to the baseline level, but instead stabilizes at approximately 150% of baseline. This may be evidence for the presence of long-term sensitization in this response.

The gradual increase in the strength of sensitization over the course of the first 5 minutes does not appear in the model. This observation may indicate that the dynamics of the cyclic AMP-dependent cascade do, in fact, produce a significant delay. A more accurate model would include two or more stages in the chemical cascade from the serotonin receptor to the K^+channel.

As is the case with habituation, there is some disagreement between experiments on the duration of the period over which the cyclic AMP concentration is elevated, and the corresponding duration of the sensitization response.

Associative Learning

Most of the inaccuracies in the modelling of associative learning could be resolved by increasing the time constant for the cyclic AMP concentration decay. The experimentally observed value of this parameter is reported as anywhere from about twenty minutes to an hour in different experiments. A time constant of an hour or more would be necessary to match data on the persistence of associative learning, whereas the time constant measured in sensitization experiments is around ten to twenty minutes. Part of this difference may be explained by the slow onset of long-term learning over the time period in which the short-term response decays. However, if the presence of a Ca^{++}-calmodulin bond increases the duration of adenylate cyclase activity as well as its amplitude, this could also explain the increased time constant observed with associative learning.

In Experiment 3, the strength of associative learning appears to stabilize at around

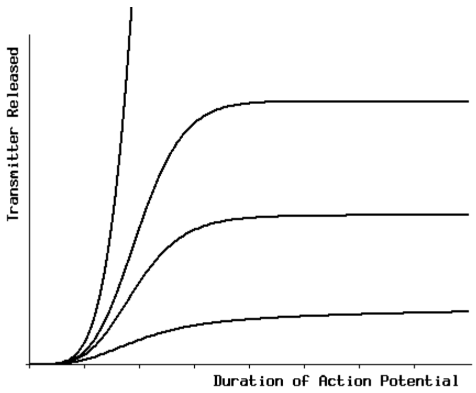


Figure 2-12: Effect of vesicle depletion on transmitter release. Each curve is for a different value of $[\mathrm{V}](t_0)$; the value of $k_1[\mathrm{V}](t_0)$ is kept constant.

300% of the baseline reflex strength. The model presented in [8] matches this result. They hypothesize that this is because a steady-state concentration of cyclic AMP is reached. This would result if the decay in cyclic AMP concentration between trials matches the influx during a trial.

The parameters used in this model for cyclic AMP production and decay, which are based directly on experimental data, will saturate at a point where the action potential duration is approximately three times as long as the baseline duration. This would produce saturation of the motor response at 300% *only if* a linear relationship between Ca^{++}influx and transmitter release is used. However, as explained in Section 2.2.4, experimental evidence suggests that this relationship is much steeper.

As the duration increases, the relationship between it and transmitter release will gradually become linear; however, a 200% increase in transmitter release occurs with about a 25% increase in action potential duration, which is far from saturating either this reaction or the cyclic AMP production process.

Another obvious candidate for explaining the saturation of the associative response is depletion of the releasable pool of vesicles. In this model, the vesicle pool is assumed to be of constant size, so this effect is not modelled. Figure 2-12 shows the effect of vesicle depletion on transmitter release, for different initial concentrations of vesicles.

2.2.4 Derivation of Chemical Interactions

The details of the chemical reactions involved in associative learning have a significant effect on the observed macroscopic behavior. Two of these reactions are analyzed below.

Calcium Influx and Transmitter Release

A 20% increase in the duration of the action potential can double the size of the EPSP [1, page 224]. This result is incompatible with a linear dependency on duration. With the fifth order dependency used in this simulation, the maximum EPSP amplitude in the experiment shown in Figure 10(a) requires only a 35% increase in action potential duration to obtain, whereas with a linear dependency a more than fourfold increase would be required.

The dependency of transmitter release on Ca^{++} influx is modelled as fourth order,[18] as described in [27].[19] This makes the synapse highly sensitive to small changes in the Ca^{++} concentration during an action potential.

To establish the nature of the dependency between action potential duration, Ca^{++} influx, and transmitter release, we must make certain assumptions about the dynamics of the underlying chemical reactions. The simplest form for modelling a fourth-order chemical reaction is:

$$4\mathrm{Ca}^{++} + \mathrm{V} \xrightarrow{k} \mathrm{R} \tag{2.11}$$

where V is one vesicle of transmitter, and R is one vesicle of transmitter released into the synaptic cleft. The corresponding differential equation is

$$\frac{d[\mathrm{R}](\tau)}{d\tau} = k \cdot [\mathrm{V}](\tau) \cdot ([\mathrm{I}_{\mathrm{Ca}}](\tau))^4 \tag{2.12}$$

[18] That is, four Ca^{++} ions must bond with each vesicle of transmitter in order to release it into the synaptic cleft.

[19] Both [8] and [20] use a simple linear dependency.

The variable τ is used to represent the (continuous) time period in which an action potential occurs. It is important to distinguish this from the discrete time t at which it appears in the simulation as an atomic event.

As vesicles are released into the synaptic cleft, the vesicle concentration $[\mathrm{V}](\tau)$ will go down, but we assume here that it remains relatively constant over the course of a single action potential.[20]

The Ca^{++} concentration during a single action potential is:

$$[\mathrm{I_{Ca}}](\tau) = \begin{cases} [\mathrm{I_{Ca}}](t) & 0 < t < \mathrm{D}(t) \\ 0 & \text{otherwise} \end{cases} \tag{2.13}$$

and the solution for transmitter release is therefore:

$$\begin{aligned} [\mathrm{R}](t) &= \int_0^{\mathrm{D}}(t)k[\mathrm{V}] \cdot ([\mathrm{I_{Ca}}](t))^4 \, d\tau \\ &= k[\mathrm{V}] \cdot ([\mathrm{I_{Ca}}](t))^4 \cdot \mathrm{D}(t) \end{aligned} \tag{2.14}$$

This equation, though it is fourth order with respect to the Ca^{++} concentration, is linear with respect to the action potential duration: this result does not give us the observed sensitivity to small changes in action potential duration.

This apparent contradiction can be resolved, however, by modelling the chemical reaction at a greater level of detail. The hypothesized mechanism for transmitter release involves four bonding sites between Ca^{++} and each vesicle of transmitter; if all four sites are filled simultaneously, the vesicle will be released. This reaction has several intermediate forms, with vesicles forming 1, 2, or 3 Ca^{++} bonds. Transmitter will be released when a single vesicle has all four bonding sites filled. If we assume that the four sites form bonds independently, then the probability of transmitter

[20] The reduction in $[\mathrm{V}](t)$ does, however, play a role in synaptic depression after extended activity [8], and may explain part of the mismatch between the behavior of this model and the experimental results shown in Figure 2-11.

release from a vesicle V, with four binding sites $\{V_1..V_4\}$, is

$$\begin{aligned}
\mathbf{P}[V_i \textit{ filled}] &= \frac{[\mathrm{V_f}]}{[\mathrm{V_f}] + [\mathrm{V_o}]} = \frac{[\mathrm{V_f}]}{4[\mathrm{V}]} && (2.15)\\
\mathbf{P}[\mathrm{R}] &= \mathbf{P}[V_1 \textit{ filled}] \wedge \mathbf{P}[V_2 \textit{ filled}] \wedge \mathbf{P}[V_3 \textit{ filled}] \wedge \mathbf{P}[V_4 \textit{ filled}] \\
&= \left(\frac{[\mathrm{V_f}]}{4[\mathrm{V}]}\right)^4 && (2.16)
\end{aligned}$$

Averaging over the population of vesicles, we get the following equation for transmitter release:

$$\begin{aligned}
\frac{d[\mathrm{R}](\tau)}{d\tau} &= \left(\frac{[\mathrm{V_f}](\tau)}{4[\mathrm{V}]}\right)^4 && (2.17)\\
[\mathrm{R}](t) &= \int_0^{\mathrm{D}(t)} \left(\frac{[\mathrm{V_f}](\tau)}{4[\mathrm{V}]}\right)^4 d\tau && (2.18)
\end{aligned}$$

where $[\mathrm{V_o}]$ is the concentration of open bonding sites, and $[\mathrm{V_f}]$ is the concentration of filled bonding sites. The bonding reaction can be described by the following equation:

$$\mathrm{Ca^{++}} + \mathrm{V_o} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{V_f} \qquad (2.19)$$

where $\mathrm{V_o}$ is one open bonding site, and $\mathrm{V_f}$ is one filled site. The differential equations are

$$\begin{aligned}
\frac{d}{d\tau}[\mathrm{V_f}](\tau) &= k_1[\mathrm{I_{Ca}}](t) \cdot [\mathrm{V_o}](\tau) - k_2 \cdot [\mathrm{V_f}](\tau) && (2.20)\\
\frac{d}{d\tau}[\mathrm{V_o}](\tau) &= -[\mathrm{V_f}](\tau) && (2.21)
\end{aligned}$$

If we assume that all bonds $\mathrm{V_f}$ decay between one action potential and the next, then $[\mathrm{V_f}](0) = 0$, $[\mathrm{V_o}](0) = 4[\mathrm{V}]$, and $[\mathrm{V_o}](\tau) = 4[\mathrm{V}] - [\mathrm{V_f}](\tau)$. In this case, the equation for $[\mathrm{V_f}](\tau)$ becomes

$$\begin{aligned} \frac{d}{d\tau}[\mathrm{V_f}](\tau) &= 4k_1[\mathrm{V}]\,[\mathrm{I_{Ca}}](t) - (k_1[\mathrm{I_{Ca}}](t) + k_2)[\mathrm{V_f}](\tau) \qquad (2.22)\\ [\mathrm{V_f}](\tau) &= \frac{4k_1[\mathrm{V}]\,[\mathrm{I_{Ca}}](t)}{k_1[\mathrm{I_{Ca}}](t) + k_2}(1 - e^{-(k_1[\mathrm{I_{Ca}}](t)+k_2)\tau}) \qquad (2.23) \end{aligned}$$

and the transmitter released during an action potential is

$$\begin{aligned} [\mathrm{R}](t) &= \int_0^{\mathrm{D}(t)} \left(\frac{[\mathrm{V_f}](\tau)}{4[\mathrm{V}]}\right)^4 d\tau \\ &= \left(\frac{k_1[\mathrm{I_{Ca}}](t)}{k_1[\mathrm{I_{Ca}}](t) + k_2}\right)^4 \int_0^{\mathrm{D}(t)} (1 - e^{-(k_1[\mathrm{I_{Ca}}](t)+k_2)\tau})^4\, d\tau \qquad (2.24) \end{aligned}$$

Although it is possible to solve this equation exactly, it is more informative to look at the two limiting cases, when the time constant $(k_1[\mathrm{I_{Ca}}](t) + k_2)^{-1} \ll \mathrm{D}(t)$ and when $(k_1[\mathrm{I_{Ca}}](t)+k_2)^{-1} \gg \mathrm{D}(t)$. In the former case, $[\mathrm{V_f}](\tau)$ will saturate very quickly relative to the duration of the action potential, and can be approximated as constant. Equation 2.24 then becomes:

$$\begin{aligned} [\mathrm{R}](t) &\approx \left(\frac{k_1[\mathrm{I_{Ca}}](t)}{k_1[\mathrm{I_{Ca}}](t) + k_2}\right)^4 \int_0^{\mathrm{D}} (t) d\tau \\ &= \left(\frac{k_1[\mathrm{I_{Ca}}](t)}{k_1[\mathrm{I_{Ca}}](t) + k_2}\right)^4 \cdot \mathrm{D}(t) \qquad (2.25) \end{aligned}$$

This corresponds to the case where bonds either form or decay very quickly. Note that the dependence of transmitter release on $[\mathrm{I_{Ca}}](t)$ in this case depends on the relative speed of the two reactions; if $[\mathrm{I_{Ca}}] \ll k_2/k_1$, then transmitter release is fourth order relative to $[\mathrm{I_{Ca}}]$ (this corresponds to the solution given in Equation 2.14), whereas