until it reaches the equilibrium state where all weights are the same.

Chapter 5

Summary and Conclusions

Most computational models of neurons have assumed that their electrical characteristics are of paramount importance. However, as the examples in this document show, chemical as well as electrical mechanisms play important computational roles in neurons. Many different mechanisms, both electrical and chemical (and, in some cases, mechanical) interact to allow the nervous system to respond to the environment in an "intelligent" manner. To adequately model the computational behavior of neurons, it is therefore necessary to consider chemical properties as well as electrical ones.

The processes underlying different aspects of classical conditioning utilize a variety of electrical and chemical processes. A functional division can also be made between short-term and long-term forms of these learning phenomena. Initial responses to conditioning experiments are short-term, but long-term mechanisms gradually come into play if learning trials are repeated over an extended time period.

The *Aplysia* system exhibits a variety of different chemical mechanisms which act at different time scales to modulate a neuron's responses to electrical stimulation. These chemical mechanisms may be localized within a single synapse, which provides neurons with a very fine-grained control over its internal state and, as a result, over its electrical responses.

These internal state "variables" have a natural time period over which they affect the cell's function, and different processes in the cell have different temporal properties. Their effects can be controlled by interactions between inputs of different types (e.g. utilizing different neurotransmitters), as well as by individual inputs.

The observed high-level learning and behavioral effects may depend on the dynamics of underlying chemical reactions.

Because they allow the possibility of responding to changes in short-term internal state, the presence of these chemical mechanisms means that a neuron's computational properties may resemble those of a finite state machine more than a combinational logic unit. This is in stark contrast to current computational models of neurons, which have little or no internal state outside the permanent state changes modelled in long-term learning processes. Some models include very short-term effects such as those which are caused by the membrane capacitance, but these generally provide little more than a capacity for short-term temporal integration.

Individual neurons are therefore much more complex than is traditionally assumed, and have the capacity to implement complex computational functions. These computations are specialized for different purposes in different regions of the brain.

In addition to mediating short-term and long-term changes in a cell's electrical behavior, chemical processes play important roles in creating the brain's anatomical structure during development. In the developing visual system, activity correlation and chemical markers cooperate to create a topographic mapping between different structures in the visual input pathway: neither mechanism alone is sufficient. Without the initial establishment of a rough topology, activity-dependent mechanisms fall prey to a variety of problems and instabilities, and the chemical processes alone cannot easily provide the precision necessary for the final map.

The pre-existing structure of a neural network, such as that specified by chemical markers in the visual system, thus directs learning in ways that vastly increase its efficiency. The network's structure, conversely, is determined in part by its inputs and the learning mechanisms that act within it. The behavior of a neural system over time results from a complex interaction between the environment, the network structure, and the nature of the learning mechanisms.

Appendix A

Modelling of Neurons

Neurons play a crucial role in the processing of sensory signals and production of behavioral responses. It is generally assumed that these functions are mediated by their electrical activity, which is described in this appendix. There are many types of neurons, and their properties vary considerably, but they all share the same basic structure. This appendix is mostly about what neurons have in common, but I also discuss some of the simpler variations. See [27] for a more comprehensive discussion of this topic.

A.1 Theme

A.1.1 Basic Electrical Properties

The first neuron studied in detail was the squid giant axon, which is a popular experimental preparation due to its large size (it is visible to the naked eye). This meant it was possible to do intracellular recordings from this neuron, even when using 1930's electrode technology. Our basic understanding of the electrical properties of neurons is derived from this example.

A neuron, like all cells, consists of a lipid membrane which separates the salt water in our bodies into intracellular and extracellular compartments. Ions (charged particles, such as sodium (Na^+) and potassium (K^+)) diffuse freely in water, but the lipid acts as an insulator, and prevents ions from flowing between the two compartments. Electrically, therefore, the cell membrane behaves as a capacitor, as structurally it consists of a thin layer of insulating material sandwiched between two conductive layers.

Ions are able to flow across the cell membrane through ion channels, which are proteins that span the membrane and create channels that are often specific to a particular type of ion. These channels act as variable resistors, where the channel resistance is controlled by such things as the voltage across the cell membrane or the presence of substances such as neurotransmitters. In addition to the ion channels, the cell has ion pumps, which actively maintain a concentration gradient of particular types of ions across the cell membrane.

When an ion channel is "open", that is, permeable to a particular ion type, there are two forces which control the flux of that ion across the membrane. Diffusion causes ions to flow down their concentration gradient, and the voltage across the membrane attracts positively charged ions toward the negative side and negatively charged ions toward the positive side of the membrane. Because of the ion pumps, which maintain the concentration gradient at a near-constant level (the ion fluxes are very small compared to their total concentration), each type of ion therefore has an equilibrium voltage, called the *reversal potential*, at which the diffusion force exactly counters the electrical force. An open ion channel behaves, electrically, as if its resistance is connected to a battery set at the reversal potential for the ions it is permeable to.

These basic properties of the cell membrane, ion channels, and ion pumps give the neuron a great detail of control over its internal electrical state. Electrical communication between neurons, on the other hand, is more often mediated by neurotransmitters, chemicals which are released by one cell to diffuse through the extracellular fluid and bind to proteins on another cell's surface. If these receptor proteins open or close ion channels in the receiving cell, the extracellular chemical "message" will be transduced into an intracellular electrical one.

There are a number of different neurotransmitters, but each neuron only produces a single neurotransmitter. Their effects may be excitatory (that is, they have a depolarizing effect on the receiving cell) or inhibitory (hyperpolarizing the receiving cell.)

A.1.2 Anatomical and Functional Structure

Understanding how information is processed and transferred within and between neurons requires a basic understanding of their anatomical structure. The general anatomy of a neuron can be seen in Figure A-1.

Within a single neuron, there are four basic functional parts:

- The *postsynaptic terminals*, most often located in the dendrites and surface of the cell body (soma), contain receptors that transduce chemical signals from other neurons into electrical currents across the cell membrane. These changes can be excitatory (depolarizing) or inhibitory (hyperpolarizing).[1] A cell may have hundreds to hundreds of thousands of postsynaptic terminals.

- The *trigger zone*, of which there is usually only one, is located in the initial segment of the axon near the soma, initiates action potentials (that is, the neuron "fires", producing a brief voltage spike) when the electrical potential exceeds a threshold value.

- The *axon*, which acts as an active transmission line, carries the voltage spike of the action potential to its presynaptic terminals.

- The *presynaptic terminals*, located on branches of the cell's axon, transduce the action potential into a chemical signal for intercellular communication. A

[1] In its resting state, a neuron is polarized, with the intracellular fluid carrying a negative charge relative to the extracellular fluid.

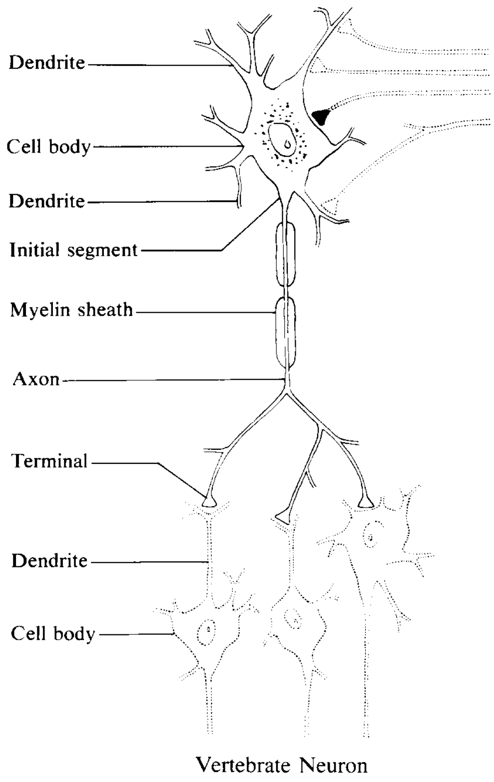

Figure A-1: What a neuron looks like (from [27], p. 97).

neurotransmitter substance is released into the extracellular fluid, and diffuses through this fluid to bind to receptors on another cell, thus completing the circuit. Like postsynaptic terminals, a single neuron may have hundreds to thousands of presynaptic terminals.

A *synapse* is a very important functional unit involving two cells: it consists of a presynaptic terminal from one cell and a postsynaptic terminal from the other. These terminals are paired, with a narrow gap called the *synaptic cleft* between them.

The dendrites are assumed to be the major "input" region of the cell, where signals from other cells are received and processed. Electrical signals travel along these long thin processes[2] until they reach the cell body, where they are integrated to produce a single electrical signal. If the cell is sufficiently depolarized by its inputs, it will fire, producing an all-or-nothing output signal which is transmitted down the axon to the presynaptic terminals. This is considered to be the source of a cell's "outputs."

A.1.3 Mechanisms of Action Potential Generation

In its resting state, the intracellular fluid carries a negative charge relative to the extracellular fluid. The resting potential of a cell is generally around -70 mV. During an action potential, this voltage is briefly reversed.

Two major ion types participate in the generation of action potentials, sodium (Na^+) and potassium (K^+). Na^+ is normally highly concentrated in the extracellular fluid, so when Na^+ channels open, both the concentration gradient and the electrical gradient tend to draw ions into the cell. This has a depolarizing action, with a reversal potential at around +55 mV.

K^+ on the other hand, is highly concentrated inside the cell, so when K^+ channels are open the ions generally flow out of the cell, which has a hyperpolarizing action. The reversal potential for K^+ is around -75 mV.

[2] A process, as defined in Webster's Dictionary, is "a prominent or projecting part of an organism or organic structure."

There are several different ion channels for each of these two important ions, each of which opens and closes under different circumstances. A highly conductive type of Na^+ channel, located along the axon, is voltage-sensitive and transient, opening briefly when the potential depolarizes to some threshold value. Na^+ channel opening initiates a positive feedback process whereby Na^+ influx further depolarizes the cell and activates more Na^+ channels. This process causes the rapid depolarization that characterizes the start of an action potential, and the effect spreads in a fast traveling wave down the cell's axon.

Voltage-activated K^+ channels then open, with a slight delay, which repolarizes the cell as the Na^+ channels close. This produces the rapid falling edge at the end of an action potential. The K^+ channels remain open somewhat longer, causing an "after-hyperpolarization" of the cell which limits the maximum frequency at which it can fire. Ion pumps restore the resting concentration of Na^+ and K^+ between action potentials.

The voltage spike at each region along the axon brings the next region above threshold, so the action potential propagates. However, because the waveform at each point results from the local properties of ion channels, local changes in its shape can be caused by locally introducing chemicals that affect those channels. For example, in the presynaptic terminals of *Aplysia*'s sensory neurons, a type of K^+ channel is partially inactivated during sensitization, which lengthens the action potential's duration. The effect of this local manipulation of the action potential waveform is discussed in Section 2.1.3.

When an action potential arrives at a presynaptic terminal, a class of voltage-sensitive calcium (Ca^{++}) channel opens. Ca^{++} is normally maintained at high concentrations outside the cell, so this causes Ca^{++} to flow into the cell. Ca^{++} influx mediates neurotransmitter release from the presynaptic terminals.

The neurotransmitter in a presynaptic terminal is contained in *vesicles*, which are tiny "bubbles" of lipid membrane. When Ca^{++} is present in the terminal, it binds to these vesicles and catalyzes a reaction by which they are attached to release sites and emptied into the synaptic gap. Active mechanisms remove free Ca^{++} from the terminal very rapidly after an action potential has passed through, so neurotransmitter is released in a brief pulse.

After their release, neurotransmitter chemicals are removed from the synaptic cleft by reuptake mechanisms which gather them back into the presynaptic terminal, or by enzymes which metabolize them, breaking them down into substances which are inert with respect to intercellular communication.

A.2 Variations

A.2.1 on the Synapse

A synapse consists of a paired presynaptic and postsynaptic terminal. In addition to *axodendritic* (that is, from the axon to the dendrite) and *axosomatic* (from the axon to the cell body) synapses, described above, some cells have *axoaxonal* synapses, where the postsynaptic terminal is on the axon of a cell, rather than on the dendrites. This has the effect of modulating a neurons output directly, instead of through the process of signal integration in the soma. One functional implication of this is that it is a much finer-grained type of control over a neuron's behavior, as it can affect individual synaptic outputs independently. An example of this can be seen in dopamine release in the striatum [56, 10], where local interactions at the presynaptic terminals can cause neurotransmitter release that is completely independent of the neuron's electrical response at the soma. This release can occur after the cell's axon is cut, at which point it no longer even has a soma.

Invertebrates have long been known to have direct electrical coupling between neurons, through a type of synapse called a *gap junction.* These junctions are comprised of coupled ion channels which span the gap between two cells, forming a direct connection between their intracellular compartments. Electrically, gap junctions create a simple resistance between the voltages of the two cells. It was long believed that gap junctions did not exist in vertebrates, but evidence has recently been found for their presence in mammals during development [54].

A.2.2 on the Inputs

In sensory neurons, specialized input regions transduce sensory signals into electrical potential changes. For example, in the retina of the eye, neurons have specialized processes called *photoreceptors*, which detect impinging photons and emit electrical signals in response. Touch sensors convert pressure and vibration into electrical signals, and proprioceptive sensors in muscles detect and signal in response to muscle tension, stretch and velocity. The firing frequency of these neurons is usually proportional to either the strength of the input or the time derivative of input strength.

In simple models of neurons, it is assumed that inputs are simply integrated in the soma to produce an electrical depolarization proportional to their sum. However, the positioning of postsynaptic terminals along the dendrites may have functional effects. For example, an inhibitory synapse may have a shunting effect on excitatory signals, which will inhibit an excitatory signal much more effectively if it is located along the section of dendrite which lies between the excitatory synapse and the soma. This may result in a neuronal response which is proportional to a product of inputs, or some combination of sums and products, instead of a simple integration [35].

In addition to *ionotropic* neurotransmitter receptors, which open and close ion channels in response to inputs, there is also a class of receptors whose action is *metabotropic*. These receptors initiate chemical processes inside the cell, instead of acting through electrical currents. The actions of neurotransmitter receptors are discussed in more detail in Appendix B.

A.2.3 on the Outputs

In addition to electrical currents caused by synaptic inputs, many neurons have intrinsic currents that cause them to fire spontaneously even in the absence of input activity [3]. These autoactive neurons show four basic firing patterns: silent (not autoactive), regular pulses at some frequency, regular bursts of pulses at some frequency, and irregular pulses. This kind of periodic activity in thalamic neurons is responsible for the brain waves seen in EEG recordings [24].

In the basic neuron model, it is assumed that action potentials propagate globally to all presynaptic terminals. However, a model has been proposed [55] where branch points in the axon may selectively block action potentials based on the interpulse interval, thus performing a more complex output function.

A.3 Recapitulation

This appendix describes the basic properties of neurons. Electrical activity within neurons and chemical signalling between neurons are generally assumed to be the mediators of a neuron's functional properties.

Although most neurons have the same basic functional components, the details of their structure and behavior vary considerably, and neurons in different brain regions can be highly specialized.

Several aspects of cellular and neural behavior are discussed in more detail in other appendices.

Appendix B

Neurotransmitter Receptors

Neurotransmitter receptors fall into two major classes, ionotropic receptors and metabotropic receptors. There are also receptors such as the NMDA receptor (described below) which have both ionotropic and metabotropic effects.

Many of the examples discussed in this appendix are glutamate receptors, as different receptors for glutamate exhibit the full range of receptor types. Glutamate is the primary excitatory neurotransmitter in the mammalian cortex, and the synapses which exhibit long-term potentiation are glutamatergic. A review can be found in [49].

B.1 Ionotropic Receptors

The best-known and most well-understood neurotransmitter receptors are ionotropic. Ionotropic receptors are ion channel proteins whose activation is regulated by a neurotransmitter binding site. Ionotropic receptors respond to specific neurotransmitters, such as glutamate, by either opening or closing their ion channels. The postsynaptic effect of ionotropic receptor activation is a rapid and brief change in the electrical currents entering or leaving the cell.

The glutamatergic AMPA receptor is a highly-conductive Na^+ channel which opens in response to the application of glutamate. It is the primary mediator of the depolarizing effects of this excitatory neurotransmitter. Postsynaptic terminals in which AMPA receptors are present usually also have other glutamate receptors, which mediate other postsynaptic effects.

B.2 Metabotropic Receptors

Metabotropic receptors have no direct effect on ion channels or membrane potential. Instead, they are coupled to metabolic processes which have indirect effects both on ion channel activity and on a variety of other cellular functions. Examples are the metabotropic serotonin receptor, described in Chapter 2, the dopamine D1 and D2 receptors, which play an important role in motor control [32, 46], and the recently discovered metabotropic glutamate receptor [58], whose activation in the hippocampus may act to filter incoming signals through selective inhibition or enhancement of the cell's electrical response. It has been hypothesized to play a role in regulating attention, and in the control of long-term potentiation.

Many metabotropic receptors are coupled to members of a family of GTP-dependent proteins (G proteins). When the receptor is activated, the associated G protein complex breaks apart into independent subunits which go on to catalyze reactions in the cell. These subunits may in some cases act directly on ion channels, producing electrical changes in the cell that persist somewhat longer than those of ionotropic receptors. However, they also regulate the cell's activity via second messenger systems, by activating enzymes such as adenylate cyclase.

G protein activation of adenylate cyclase stimulates the production of cyclic AMP, which controls cellular processes by activating a cyclic AMP-dependent protein kinase. Protein kinases regulate the activity of a variety of substrate proteins through phosphorylation (described in Appendix C.)

Metabotropic receptors act on a much slower time scale than ionotropic receptors, due to the complex cascade of chemical events that mediate their effects. This cascade also acts to amplify the incoming signal. For example, a small amount of activated G protein can produce a large increase in the cyclic AMP concentration through activation of adenylate cyclase. The effects of metabotropic receptors may also persist over an extended time period, in part through the catalytic action of protein kinases.

Activation of metabotropic receptors can have persistent and far-reaching effects, ranging from short-lived changes in the sensitivity of other receptors or ion channels to large-scale morphological change.

B.3 The NMDA Receptor

Some receptor types, such as the glutamatergic NMDA receptor, have both ionotropic and metabotropic actions. NMDA receptor activation is necessary for the induction of long-term potentiation, a form of synaptic change that requires coactivation of presynaptic and postsynaptic cells.

NMDA receptors contain an ion channel that is primarily permeable to calcium ions. Ca^{++} influx has both a direct, rapid electrical effect on the cell, and a range of indirect metabolic effects.[1]

If glutamate binds to an NMDA receptor when the cell is near its resting potential, the ion channel is blocked by magnesium ions (Mg^{+}), which, like Ca^{++} are present in high concentrations outside the cell. Magnesium, however, is unable to fit through this channel and enter the cell. When the cell depolarizes, Mg^{+} loses its affinity to the channel, and it is freed up for use by Ca^{++} ions. Maximum channel activation occurs around -20 to -30 mV. Less Ca^{++} influx is seen at higher voltages because they are nearer its reversal potential, and less influx is seen at lower voltages due to the Mg^{+} block.

The effect of the NMDA receptor is therefore highly nonlinear. They exhibit almost no activity at low voltage, but as the cell is sufficiently excited by other receptors

[1] Ca^{++} is extremely important in regulating many aspects of neuronal function. It plays some role in all of the processes discussed in these appendices.

(for example, the AMPA receptor, which is usually present in the same postsynaptic terminals), it will "turn on", enhancing the depolarizing effect of strong input signals, and initiating longer-term processes in the cell. This dependence on presynaptic activity coupled with postsynaptic depolarization, combined with its ability to produce long-term changes through the second-messenger effects of Ca^{++} ions, give the NMDA receptor a unique and important role in neuronal functioning.

B.4 The Importance of Calcium in Neurons

Calcium mediates many important intracellular processes. As a charged ion, the flux of Ca^{++} ions across the cell membrane has a direct electrical effect, acting to depolarize the cell. However, unlike Na^{+} and K^{+} ions, whose primary effects appear to be electrical, Ca^{++} also mediates many important metabolic processes inside the cell. Ca^{++} ions are necessary to induce transmitter release from vesicles at presynaptic terminals. They also act, in the form of Ca^{++}-calmodulin, as an important cellular second messenger, activating a class of calcium-dependent protein kinases. Ca^{++} ions have also received a great deal of attention for their role in the induction of long-term potentiation. Some of these processes are discussed in more detail in Appendices C and D.

B.5 Sources of Calcium

A knowledge of the sources of Ca^{++} in the cell is important for understanding the control of Ca^{++}-dependent processes. This section provides a basic introduction to Ca^{++}sources.[2]

The steady-state intracellular calcium concentration is around 10^{-7} M. Most ion channels are inactivated at concentrations above 10^{-6} M., which defines a physiological range of concentrations at which biologically meaningful events occur.

There are two major sources of intracellular calcium: influx through ion channels in the cell membrane, and release from internal calcium stores in the endoplasmic reticulum or other locations.

[2] This information is taken from a number of sources, including [4, 39, 64].

B.5.1 Voltage-dependent Calcium Channels

There are four major types of calcium channels that operate in a voltage-dependent manner, opening in response to cell depolarization. The activity of ion channels is defined in terms of their conductance, their location in the cell membrane, and the conditions under which they open and close. Two voltage-activated calcium channels, the T-type (transient) and L-type (long-lasting) channels, are discussed here.

T-type calcium channels are low-voltage-activated and transient. These channels decay rapidly to an inactive state when cell depolarization is prolonged, and become insensitive to further changes in voltage until the cell repolarizes to a level that is outside their inactivation range, at which point they return to a resting (closed) state. They are believed to be important in producing the spontaneous oscillatory activity that underlies periodic bursting in the thalamus. T-type calcium channels may also play a role in mossy fiber LTP.

The L-type calcium channel is one of several high-voltage-activated channels. It, too, inactivates under sustained depolarization, but its time constant is extremely long, so under most conditions it can be considered a purely voltage-dependent channel. The L-type channel is activated in response to large changes in membrane potential, such as occur during action potentials. It is widely distributed in the central nervous system and tends to be concentrated in cell bodies and proximal dendrites, especially at the base of the major dendrites. As such, it is believed to play a role in action potential generation.

The L-type channel must be phosphorylated before it can open; this process can be mediated by a cyclic AMP-dependent protein kinase. There is evidence to indicate that its activation may be actively modulated by endogenous substances.[3] The mechanism for Ca^{++} channel modulation is likely to involve neurotransmitters or peptides whose receptors are coupled to G proteins. These proteins may affect the channel

[3] Endogenous substances are those which occur naturally in the body.

directly or act via an intracellular second messenger such as cyclic AMP.[4]

B.5.2 The NMDA Receptor

The NMDA (for n-methyl-d-aspartate, a drug which activates it) receptor is a neurotransmitter receptor which has a voltage-dependent Ca^{++} channel. Unlike the T-type and L-type channels, the NMDA receptor requires the presence of glutamate, an excitatory neurotransmitter, for its activation. This receptor is described in Appendix B.

B.5.3 Calcium Release from Internal Stores

Increases in calcium concentration in the cell generally last only a few milliseconds. Calcium is rapidly removed from the cell, bound up in protein complexes such as Ca^{++}-calmodulin, or taken up into internal stores, which may be in mitochondria, in the endoplasmic reticulum, or in calciosomes.

Calcium release from internal stores can be induced by caffeine. The function of this release may be to induce oscillatory activity in the cell; it is probably not involved in transmitter release.

Calcium release from the endoplasmic reticulum is also induced by a chemical called inositol 1,4,5-triphosphate ($InsP_3$). The intracellular concentration of $InsP_3$ increases in response to the activation of phospholipase C from cell receptors. This is a relatively recently discovered form of cellular second messenger, and its action is not well understood. It may be the mechanism by which caffeine acts.

B.6 Calcium Actions

The particular changes mediated by Ca^{++} influx may vary from cell to cell, and may also be modulated by other substances. This section describes a few of the known

[4]See Appendices B and C for more information on G proteins, second messengers, and protein phosphorylation.

actions of Ca^{++} in neural cells.

Its second-messenger effects and role in long-term potentiation are discussed in other appendices, so this section will focus on electrical effects and on the role of Ca^{++} in transmitter release.

B.6.1 Electrical Effects

Many of Ca^{++}'s actions in cells are initiated by Ca^{++} influx through ion channels in the cell membrane. Because it is a positively charged ion, this influx results in a voltage change in the cell. There is some controversy over the relative importance of electrical and metabolic Ca^{++} effects.

One of the more striking properties of voltage-activated Ca^{++} channels is that, like the Na^{+} channels which mediate the depolarizing phase of action potentials, their opening produces a positive feedback response, which can produce Ca^{++} spikes in the cell. Ca^{++} spikes can occur, for example, in a cell's dendrites in response to input-mediated depolarization. They are much slower and smaller in amplitude than Na^{+} spikes.

In addition to calcium spiking due to voltage-activated Ca^{++} channels, the voltage-sensitivity of NMDA channels can result in a nonlinear dendritic response to synaptic inputs.

Dendrites have traditionally been considered to be passive electrical cables whose primary role was the transport of electrical signals into the cell body for integration in the trigger zone, but the presence of depolarization-induced dendritic Ca^{++} spikes indicates that they may play a more active in information processing than has been traditionally hypothesized. The properties of active dendrites have been modelled and found to have a significant effect on the cell's response to patterns of synaptic input with differing spatial distributions [44, 43].

Low-voltage-activated Ca^{++} channels also play a role in generating periodic oscillations in spontaneously active cells. The low activation threshold of these channels produces a small "leak" current, which slowly depolarizes the cell. As it depolarizes, more Ca^{++} channels are activated, which brings the cell to its firing threshold, at which point the Na^{+} channels take over and an action potential is generated. These Ca^{++} channels are transient, but the hyperpolarization of the cell due to K^{+} channel opening at the end of an action potential re-activates them, and the process repeats at some characteristic frequency.

Periodic oscillations in cells are important in a class of neural circuits called *central pattern generators*, which control periodic motor responses such as walking, chewing, and wing-flapping [3].

B.6.2 The Role of Calcium in Neurotransmitter Release

The presynaptic terminals of neurons are populated with voltage-activated Ca^{++} channels. When these channels open during an action potential, Ca^{++} flows rapidly into the terminal. It is removed from the terminal equally rapidly after the channels close at the end of an action potential, either by being bound to calmodulin or by a variety of other uptake mechanisms. However, during that brief period, the presence of free Ca^{++} in the terminal causes the release of neurotransmitter into the synaptic cleft [33].

Ca^{++} catalyzes a reaction between vesicles of neurotransmitter and release sites on the cell membrane. It requires four Ca^{++} ions to catalyze the release of a single vesicle of transmitter [27], through a process called *exocytosis*. Vesicles are small free-floating bubbles inside the presynaptic terminals, with neurotransmitter on the inside and a lipid membrane on the outside, made of the same substance as the cell membrane. In exocytosis, Ca^{++} ions increase the affinity between vesicles and release sites, where the lipid membrane of the vesicle can merge with the cell membrane, dumping its contents outside the cell.

Appendix C

Protein Phosphorylation

Essentially everything that happens inside a cell is mediated by proteins. The structure of proteins is what is encoded in the DNA, and the particular proteins present in a cell determine its behavior, its morphology, and its function in the body. The obvious differences between a skin cell, a muscle cell, and a nerve cell, for example, are caused by differences in which particular proteins are present and active in those cells.

The activity of many proteins can be up- or down-regulated by protein phosphorylation, an important mechanism by which extracellular messengers, such as neurotransmitters and hormones, influence what happens inside a cell. This appendix describes the basic mechanisms involved in protein phosphorylation. Much of this information was taken from [51].

C.1 First and Second Messengers

Chemical *first messengers* travel in extracellular space and bind to receptors on cell surfaces, but they do not themselves carry messages into the cell (though they may originate in and be released from cells.) Instead, their message is relayed by *second messengers*, which are produced in response to receptor activation, and which mediate the cell's response to the extracellular signal.

The time course of second messenger effects may be quite long, and they can control a wide variety of intracellular processes. Their effects can range from indirect opening or closing of ion channels, to up- or down-regulation of the activity of a variety of proteins in the cell, to modifications in gene transcription, which may result in large-scale changes in cell morphology. Many of these results are accomplished through protein phosphorylation.

C.2 What is Protein Phosphorylation?

A class of proteins, called *protein kinases*, act as catalysts for protein phosphorylation reactions. The basic reaction is the binding of a phosphate molecule to a phosphorylation site on a protein, which results in a conformational change in the protein. Many proteins respond to phosphorylation by changes in their activity. The reaction, therefore, can be seen as turning the protein "on" or "off", or increasing or decreasing the rate at which it acts.

Each of protein kinase is capable of phosphorylating a specific set of *substrate proteins*, with different affinities for each. A protein kinase may phosphorylate a wide range of proteins, or it may be specific to one or a very few.

The complementary reaction is protein dephosphorylation, which is catalyzed by a class of proteins called *protein phosphatases*. Each protein phosphatase also acts on some set of substrate proteins, but their substrate specificity is generally not complementary to any particular protein kinase.

C.3 What does it Do?

1. A number of ion channels can be activated or inactivated by protein phosphorylation. An example of this is the K^+ channel discussed in Section 2.1.3. This channel is inactivated by phosphorylation, which affects the duration of action

potentials in the cell, and indirectly affects synaptic strength due to the relationship between action potential duration and transmitter release. The L-type Ca^{++} channel, in contrast, is active only when phosphorylated [4].

2. The intermediate-term maintenance of long-term synaptic potentiation in the hippocampus (over a period lasting 3-6 hours after induction of LTP) is blocked by protein kinase inhibitors, which indicates the importance of protein phosphorylation in mediating this process. LTP discussed in detail in Appendix D.

3. DNA transcription factors may act as substrate proteins in phosphorylation reactions [12]. These proteins bind to particular DNA sequences and activate or inhibit transcription of the associated genes. The role of cyclic AMP in regulating long-term synaptic potentiation in *Aplysia*'s sensory neurons, described in Section 2.1.3, is an example of this use of protein phosphorylation.

4. Protein kinases can regulate their own actions through autophosphorylation. In the case of cyclic AMP-dependent protein kinase RII, autophosphorylation decreases the rate at which the kinase is deactivated, and thus prolongs its period of activity. Other possible actions of autophosphorylation are to change the affinity of the protein kinase for its substrate proteins, or to maintain its activity independent of the continued presence of a second messenger.

   Phosphorylation of a protein kinase by some other protein kinase could provide a mechanism by which the presence of one second messenger might modulate the cell's response to another second messenger.

5. The activity of proteins can also be indirectly regulated by regulating protein phosphatases. Several protein kinases have been found that inhibit protein phosphatase activity. This will result in prolonged phosphorylation of the substrate proteins that the phosphatase acts on (which may be distinct from those affected directly by the protein kinase.) This provides another mechanism by

which the action of one second messenger can indirectly control the cell's response to other second messengers.

6. The activity of many hormone and neurotransmitter receptors and other proteins that regulate the production of second messengers is also affected by protein phosphorylation. This is yet another mechanism by which second messengers can modulate their own activity or the activity of other second messengers.

C.4 How is it Controlled?

There are many substances which regulate protein kinase activity. Among the best-known of these are Cyclic AMP, cyclic GMP, and the compounds Ca^{++}-calmodulin and Ca^{++}-phosphatidylserine. Each of these is a cellular second messenger, whose concentration in the cell is regulated in part by first messengers such as neurotransmitters and hormones.

Cyclic AMP is produced by adenylate cyclase, and is metabolized (broken down) by phosphodiesterase. Cyclic GMP is produced by guanylate cyclase, and is also metabolized by phosphodiesterase. Each of these enzymes can itself undergo phosphorylation. Adenylate cyclase and guanylate cyclase are activated by G proteins coupled to receptors in the cell membrane. This process is described in Appendix B. The concentration of these second messengers may remain elevated for minutes to hours after the initial stimulus initiating their production.

The calcium compounds Ca^{++}-calmodulin and Ca^{++}-phosphatidylserine are formed, along with a number of less well-known second messenger compounds, whenever Ca^{++} ions are present in the intracellular fluid. This can come about either by the opening of calcium-permeable ion channels in the cell membrane or by release of calcium from internal stores.[1] Ca^{++} bonds decay rapidly, and free Ca^{++} ions are actively removed from the cell, so the concentration of these compounds peaks and

[1]See Appendix B.4 for details.

decays rapidly after the influx of Ca^{++} ends.

Appendix D

Long-Term Potentiation

D.1 Neural Plasticity and Learning

Learning in neurons is generally assumed to be mediated by changes in synaptic efficacy. These can result from changes in the signal transmitted by an existing synapse or morphological changes in which new synapses are formed or existing synapses grow or atrophy. Outside of early development, learning is usually assumed to take place primarily in previously existing synapses.

This learning can, in theory, take place either in the presynaptic terminal, which would result in changes in the amount of transmitter released per action potential, or in the postsynaptic terminal, which would result in changes in the response for a given amount of transmitter[6], or both.

Presynaptic mechanisms can be very specific, involving a single or small group of connections, as individual presynaptic terminals tend to be isolated both electrically and chemically. On the other hand, postsynaptic mechanisms can more easily affect large populations of synapses[38].

D.2 Long-Term Potentiation

Long-term potentiation (LTP), first studied in the hippocampus, is a persistent increase in the synaptic efficacy of glutamatergic synapses in response to coactivation of presynaptic and postsynaptic cells. It has been observed in many regions in the brain, and is believed to be an important mechanism for learning.

Long-term potentiation can be induced either by a burst of activity in an input pathway or by input activity coupled with excitation of the target cell from another source. The behavior of this kind of LTP is similar to the classic Hebb learning rule, where the weight of a synaptic link is enhanced after paired activity in the source and target neuron. Because of its Hebb-like properties, long-term potentiation has generated a great deal of interest in the neuroscience community. Because of this interest, our understanding of the mechanisms underlying LTP is changing rapidly. A recent review can be found in [5].

D.2.1 Stages in LTP induction and maintenance

Like the forms of learning seen in *Aplysia*, long-term potentiation has several stages, ranging from short-term potentiation which lasts about an hour, through three different stages of long-term potentiation, which depend on different types of cellular mechanisms.

Short-term potentiation, lasting 30-60 minutes, is likely to be presynaptic, and is independent of protein kinase activity.

The first phase of LTP, in contrast, requires the activity of protein kinases. This implies that protein phosphorylation is required to make the transition from short-term to long-term forms of learning in this system. In particular, Ca^{++}-phospholipid-dependent protein kinases have been implicated in the initial phase of LTP, which lasts only a couple of hours.

The second phase of LTP, lasting up to 6 hours, requires protein synthesis. The third, and longest-lasting, phase of LTP requires changes in gene transcription.

D.2.2 Properties of LTP

LTP is characterized by three basic properties. It is cooperative, meaning that it responds to the activation of multiple inputs. There there is a threshold in the strength of inputs below which LTP does not occur. Activation levels which are below the threshold for LTP induction may, however, induce short-term synaptic potentiation. It is associative, in the sense that a weak input may be potentiated by coactivation with a strong input. And it is input-specific; that is, potentiation acts at the level of individual synapses.

D.2.3 Induction of LTP

These properties are believed to be mediated by the response properties of NMDA receptors, which require paired presynaptic activity and postsynaptic depolarization for activation. Strong evidence has been found for the role of NMDA receptor activation in the induction of LTP. However, NMDA alone is not usually sufficient, and, in fact, NMDA receptors have been shown to inhibit as well as enhance LTP in different situations. It has been hypothesized that coactivation of NMDA and metabotropic glutamate receptors may be necessary for LTP induction.

Both these receptors increase intracellular Ca^{++} levels, but they act in different ways. NMDA receptor activation causes Ca^{++} influx through its ion channel, whereas metabolic glutamate receptors may affect Ca^{++} levels by increasing Ca^{++} release from internal stores. This effect is most likely mediated by the second messenger inositol 1,4,5-triphosphate ($InsP_3$).

D.2.4 Maintenance of LTP

The maintenance of LTP requires persistent changes in synaptic efficacy. The locus of these changes is not known, but both presynaptic and postsynaptic modifications have been implicated. Different stages of LTP may occur in different locations.

In the postsynaptic cell, possible mechanisms of action include sensitization of the AMPA receptors by phosphorylation in the initial phase, and possibly the synthesis of more sensitive subtypes of AMPA receptors in later phases. NMDA receptors may also be affected by phosphorylation, reducing the effectiveness of the Mg^+ block, or they may be affeced by agonists such as arachidonic acid or $InsP_3$.

Because the induction of LTP occurs in the postsynaptic terminal, presynaptic changes require the release of a retrograde messenger to communicate this activation to the presynaptic terminal. The nature of the retrograde messenger is not known, although both arachidonic acid and nitrous oxide (NO) have been hypothesized.

The mechanism for presynaptic changes is also unknown, although changes in Ca^{++} levels or in the process of transmitter release have been hypothesized. Presynaptic metabotropic glutamate receptors have been hypothesized as a possible mediator of presynaptic changes. However, which presynaptic mechanisms are most likely to be involved depends in part on the (currently unknown) identity of the retrograde messenger.

D.3 Summary

What is currently known about LTP in the mammalian nervous system implies that it acts through metabolic pathways that are very similar to the mechanisms of action of classical conditioning in *Aplysia*. These mechanisms, namely, protein phosphorylation, protein synthesis, and gene transcription, are basic metabolic processes in the cell, and the regulation of these processes by neurotransmitters provides a powerful mechanism for information processing and the control of neuronal and behavioral responses. These responses can be separated into short-term and long-term components, which can be induced or blocked separately and which utilize different metabolic pathways. The interaction between multiple mechanisms and pathways, and the synapse specificity of the responses, provides the neuron with a very fine-grained degree of control over its responsiveness to different input patterns.

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