Software Experiment #4a

HOW DO NEURONS COMMUNICATE VIA SYNAPSES?

Content areas: Basic neuroscience covered in biology, physiology, psychology, and engineering courses

Pre-requisite knowledge: Cell biology, human nervous system, first order systems

Learning Objectives: After this lesson, students should be able to:

- functioning of a synapse
- the role of synapses in communication between neurons
- the effect of drugs on disruption of communications

Time Required:

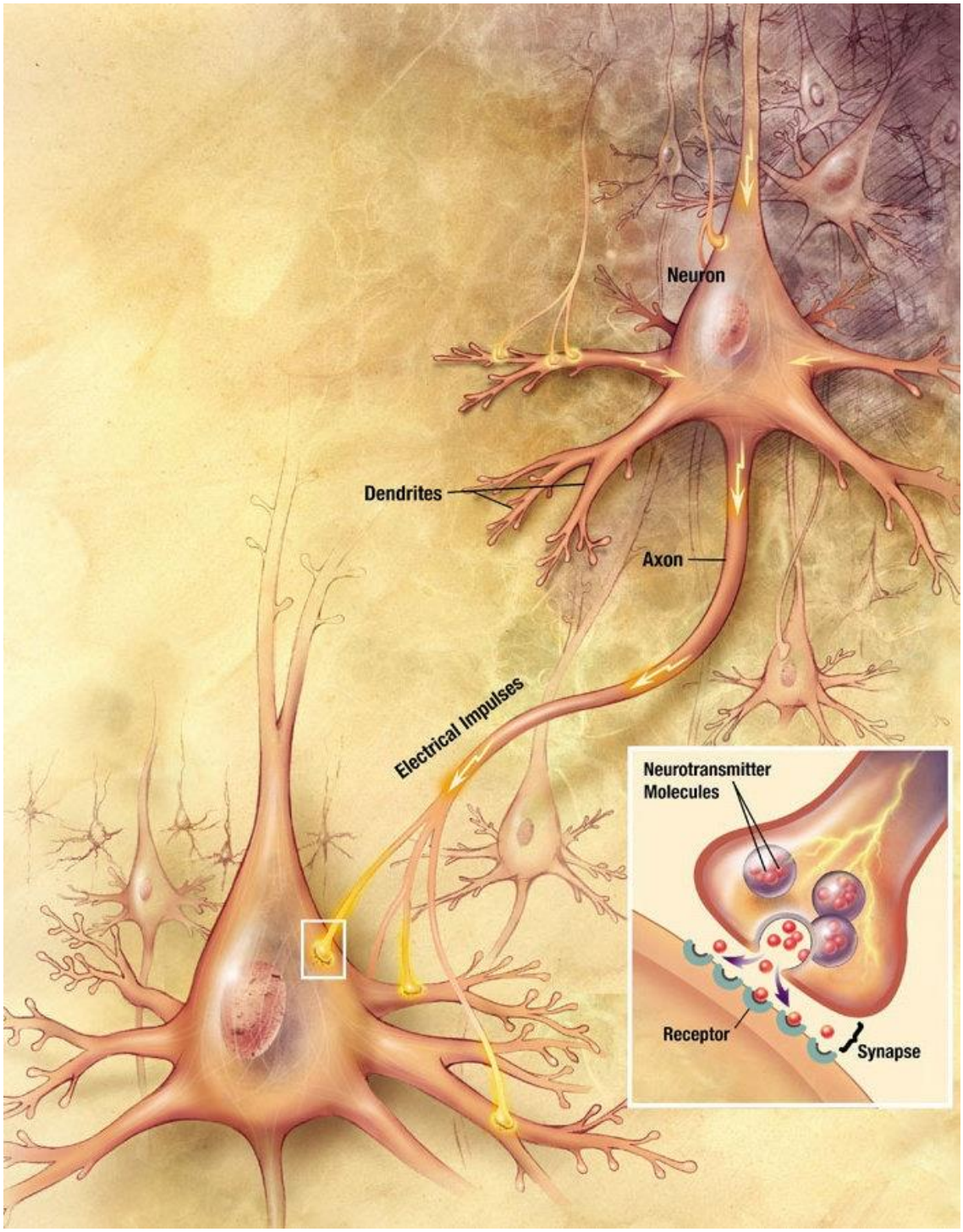
Keywords chemical synapse, neurotransmitter, pre-synapse, post-synapse

Summary This lesson builds on the material in the previous lessons on AP and bursting. An important mode of communication between cells is via chemical synapses. This lesson explain how one neuron transmits it's signal to another via a one-way path termed 'synapse'. The electrical signal is converted into a chemical signal and passed from the pre-synapse to the post-synapse. This is then reconverted into an electrical signal at the post-synaptic end.

INTRODUCTION / MOTIVATION

SYNAPSES

(adapted from http://www.biologymad.com/NervousSystem/synapses.htm and http://en.wikipedia.org/wiki/Chemical_synapse)



Chemical synapses are specialized junctions through which neurons signal to each other and to non-neuronal cells such as those in muscles or glands. Chemical synapses allow neurons to form circuits within the central nervous system. They are crucial to the biological computations that underlie perception and thought. They allow the nervous system to connect to and control other systems of the body.

The adult human brain is estimated to contain from 10^{14} to 5×10^{14} (100-500 trillion) synapses. Each mm^3 of cerebral cortex contains roughly a billion of them

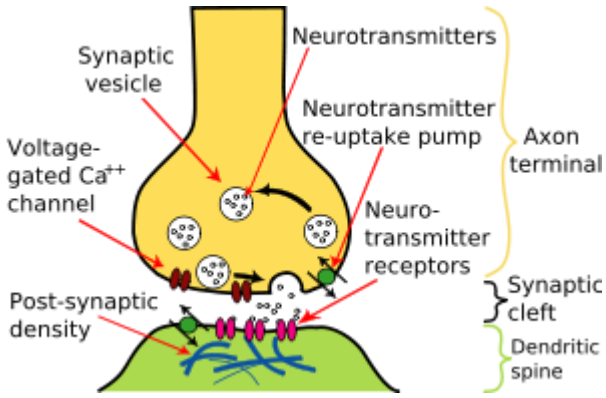
Information from one neuron flows to another neuron across a **synapse**. The synapse is a small gap separating neurons. The synapse consists of:

- a **presynaptic ending** that contains neurotransmitters, mitochondria and other cell organelles,
- a **postsynaptic ending** that contains receptor sites for neurotransmitters and,
- a **synaptic cleft** or space between the presynaptic and postsynaptic endings. It is about 20nm wide.

An action potential cannot cross the synaptic cleft between neurones. Instead the nerve impulse is carried by chemicals called **neurotransmitters**. These chemicals are made by the cell that is sending the impulse (the **pre-synaptic neurone**) and stored in **synaptic vesicles** at the end of the axon. The cell that is receiving the nerve impulse (the **post-synaptic neurone**) has chemical-gated ion channels in its membrane, called **neuroreceptors**. These have specific binding sites for the neurotransmitters.

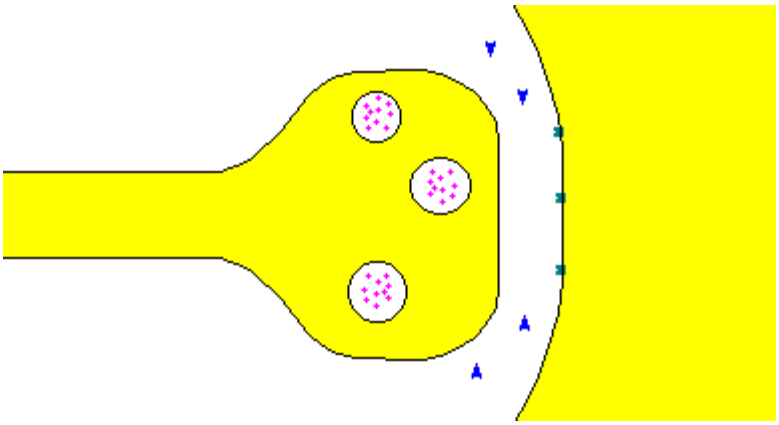
Check Point ☐ Synapse is a small gap separating neurones

- Synapses consist of:
  - **presynaptic ending** (where neurotransmitters are made)
  - **post synaptic ending** (has neuroreceptors in the membrane)
  - **synaptic cleft**
- Action potentials **cannot** cross the synaptic cleft
- Nerve impulse is carried by **neurotransmitters**



Schematic of a chemical synapse between an axon of one neuron and a dendrite of another. Synapses are specialised minute gaps between neurons. The electrical impulses arriving at the axon terminal triggers the release of packets of chemical messengers (neurotransmitters), which diffuse across the synaptic cleft to receptors on the adjacent dendrite temporarily affecting the likelihood that an electrical impulse will be triggered in the latter neuron. Once released the neurotransmitter is rapidly metabolised or is pumped back into a neuron.

1. At the end of the pre-synaptic neurone there are voltage-gated calcium channels. When an action potential reaches the synapse these channels open, causing calcium ions to flow into the cell.
2. These calcium ions cause the synaptic vesicles to fuse with the cell membrane, releasing their contents (the neurotransmitter chemicals) by exocytosis.
3. The neurotransmitters diffuse across the synaptic cleft.
4. The neurotransmitter binds to the neuroreceptors in the post-synaptic membrane, causing the channels to open. In the example shown these are sodium channels, so sodium ions flow in.
5. This causes a depolarisation of the post-synaptic cell membrane, which may initiate an action potential, if the threshold is reached.
6. The neurotransmitter is broken down by a specific enzyme in the synaptic cleft; for example the enzyme **acetylcholinesterase** breaks down the neurotransmitter **acetylcholine**. The breakdown products are absorbed by the pre-synaptic neurone by endocytosis and used to re-synthesise more neurotransmitter, using energy from the mitochondria. This stops the synapse being permanently on.



Check Point ☐ How the impulse is transmitted across the synaptic cleft

action potential reaches the presynaptic terminal
☐
voltage-gated Ca^{2+} channels open
☐
influx of Ca^{2+}
☐
synaptic vesicles fuse with membrane (exocytosis)
☐
neurotransmitters are released into synaptic cleft and diffuse to postsynaptic terminal
☐
neurotransmitter binds to neuroreceptor on postsynaptic membrane
☐
causes Na+ channels to open, and Na+ flows into postsynaptic membrane
☐
if threshold is reached then action potential is initiated
☐
neurotransmitter is broken down by specific enzymes in the synaptic cleft.

Different Types of Synapses

The human nervous system uses a number of different neurotransmitter and neuroreceptors, and they don't all work in the same way. We can group synapses into 5 types:

1. **Excitatory Ion Channel Synapses.**

   These synapses have neuroreceptors that are sodium channels. When the channels open, positive ions flow in, causing a local depolarisation and making an action potential more likely. This was the kind of synapse described above. Typical neurotransmitters are acetylcholine, glutamate or aspartate.

2. **Inhibitory Ion Channel Synapses.**

   These synapses have neuroreceptors that are chloride channels. When the channels open, negative ions flow in causing a local hyperpolarisation and making an action potential less likely. So with these synapses an impulse in one neurone can inhibit an impulse in the next. Typical neurotransmitters are glycine or GABA.

3. Non Channel Synapses.

These synapses have neuroreceptors that are not channels at all, but instead are membrane-bound enzymes. When activated by the neurotransmitter, they catalyse the production of a "messenger chemical" inside the cell, which in turn can affect many aspects of the cell's metabolism. In particular they can alter the number and sensitivity of the ion channel receptors in the same cell. These synapses are involved in slow and long-lasting responses like learning and memory. Typical neurotransmitters are adrenaline, noradrenaline (NB adrenaline is called epinephrine in America), dopamine, serotonin, endorphin, angiotensin, and acetylcholine.

4. Neuromuscular Junctions.

These are the synapses formed between motor neurones and muscle cells. They always use the neurotransmitter acetylcholine, and are always excitatory. We shall look at these when we do muscles. Motor neurones also form specialised synapses with secretory cells.

5. Electrical Synapses.

In these synapses the membranes of the two cells actually touch, and they share proteins. This allows the action potential to pass directly from one membrane to the next. They are very fast, but are quite rare, found only in the heart and the eye.

Check Point ☐ Different types of synapses

Excitatory ion channel synapses - neuroreceptors are Na+ channels. When Na+ channels open, local depolarisaition occurs, if threshold is reached then action potential is initated

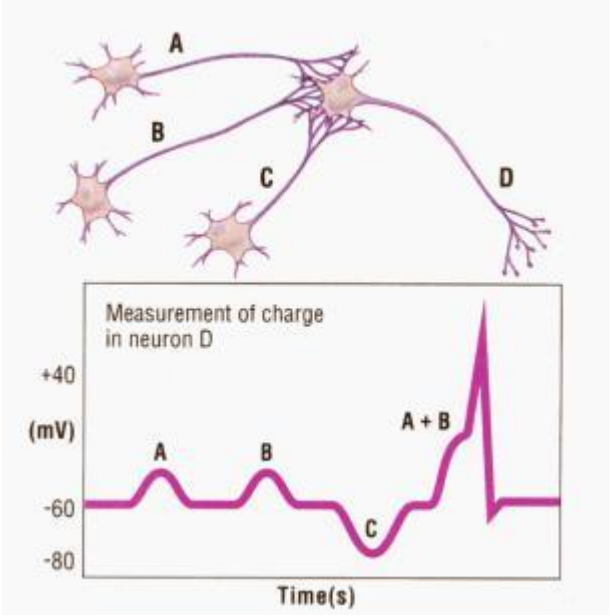
inhibitory ion channels - neuroreceptors are Cl- channels. When Cl- channels open, hyperpolarisation occurs, making action potential less likely

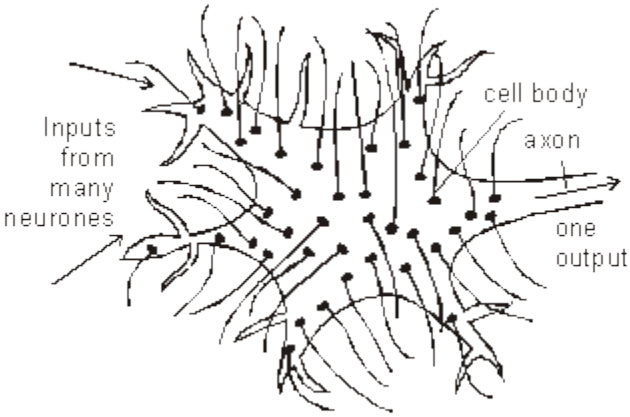
Non channel synapses - neuroreceptors are membrane-bound enzymes. When activated, they catalyse the 'messenger chemical', which in turn can affect the sensitivity of the ion channel receptors in the cell

Neuromuscular junctions - synapses formed between motor neurones and muscle cells. Always use the neurotransmitter acetylchline, and are always excitatory

Electrical synapses - the membranes of the two cells actually touch and they chare proteins. The action potential can pass directly from one membrane to the next

Summation





When one postsynaptic neuron is excited/inhibited by more than one presynaptic neuron. Thus several neurons converge and release their neurotransmitters towards one neuron.

One neurone can have thousands of synapses on its body and dendrons. So it has many inputs, but only one output. The output through the axon is called the **Grand Postsynaptic Potential** (GPP) and is the sum of all the excitatory and inhibitory potentials from all that cell's synapses. If there are more excitatory potentials than inhibitory ones then there will be a GPP, and the neurone will "fire", but if there are more inhibitory potentials than excitatory ones then there will not be a GPP and the neurone will not fire.

This summation is the basis of the processing power in the nervous system. Neurones (especially interneurones) are a bit like logic gates in a computer, where the output depends on the state of one or more inputs. By connecting enough logic gates together you can make a computer, and by connecting enough neurones together to can make a nervous system, including a human brain.

So why bother? Why have gaps in the nerves?

1. They make sure that the flow of impulses is in one direction only. This is because the vesicles containing the transmitter are only in the presynaptic membrane and the receptor molecules are only on the postsynaptic membrane.
2. They allow integration, e.g. an impulse travelling down a neurone may reach a synapse which has several post synaptic neurones, all going to different locations. The impulse can thus be dispersed. This can also work in reverse, where several impulses can converge at a synapse.
3. They allow 'summation' to occur. Synapses require the release of sufficient transmitter into the cleft in order for enough of the transmitter to bind to the postsynaptic receptors and the impulse to be generated in the postsynaptic neurone. In **spatial summation**, several presynaptic neurones converge at a synapse with a single post synaptic neurone. In **temporal summation** there is only one presynaptic and one postsynaptic neurone but the frequency of impulses reaching the synapse is important. Both types of summation allow for 'grading' of nervous response – if the stimulation affects too few

presynaptic neurones or the frequency of stimulation is too low, the impulse is not transmitted across the cleft.

4. They allow the 'filtering out' of continual unnecessary or unimportant background stimuli. If a neurone is constantly stimulated (e.g. clothes touching the skin) the synapse will not be able to renew its supply of transmitter fast enough to continue passing the impulse across the cleft. This 'fatigue' places un upper limit on the frequency of depolarisation.

Drugs and the Nervous System

(additional information, however, helpful to your understanding)

Almost all drugs taken by humans (medicinal and recreational) affect the nervous system. From our understanding of the human nervous system we can understand how many common drugs work. Drugs can affect the nervous system in various ways, shown in this table:

Drug action	Effect
Mimic a neurotransmitter	Switch on a synapse
Stimulate the release of a neurotransmitter	Switch on a synapse
Open a neuroreceptor channel	Switch on a synapse
Block a neuroreceptor channel	Switch off a synapse
Inhibit the breakdown enzyme	Switch on a synapse
Inhibit the Na^+K^+ATPase pump	Stop action potentials
Block the Na^+ or K^+ channels	Stop action potentials

Drugs that stimulate a nervous system are called **agonists**, and those that inhibit a system are called **antagonists**. By designing drugs to affect specific neurotransmitters or neuroreceptors, drugs can be targeted at different parts of the nervous system. The following paragraph describe the action of some common drugs. You do not need to know any of this, but you should be able to understand how they work.. By designing drugs to affect specific neurotransmitters or neuroreceptors, drugs can be targeted at different parts of the nervous system. The following paragraph describes the action of some common drugs. You do not need to know any of this, but you should be able to understand how they work.

1. Drugs acting on the central nervous system

In the reticular activating system (RAS) in the brain stem noradrenaline receptors are excitatory and cause wakefulness, while GABA receptors are inhibitory and cause drowsiness. Caffeine (in coffee, cocoa and cola), theophylline (in tea), amphetamines, ecstasy (MDMA) and cocaine all promote the release of noradrenaline in RAS, so are stimulants. Antidepressant drugs, such as the tricyclics, inhibit the breakdown and absorption of noradrenaline, so

extending its effect. Alcohol, benzodiazepines (e.g. mogadon, valium, librium), barbiturates, and marijuana all activate GABA receptors, causing more inhibition of RAS and so are tranquillisers, sedatives and depressants. The narcotics or opioid group of drugs, which include morphine, codeine, opium, methadone and diamorphine (heroin), all block opiate receptors, blocking transmission of pain signals in the brain and spinal cord. The brain's natural endorphins appear to have a similar action.

The brain neurotransmitter dopamine has a number of roles, including muscle control, pain inhibition and general stimulation. Some psychosis disorders such as schizophrenia and manic depression are caused by an excess of dopamine, and antipsychotic drugs are used to block the dopamine receptors and so reduce its effects. Parkinson's disease (shaking of head and limbs) is caused by too little dopamine compared to acetylcholine production in the midbrain. The balance can be restored with levodopa, which mimics dopamine, or with anticholinergic drugs (such as procyclidine), which block the muscarinic acetylcholine receptors.

Tetrodotoxin (from the Japanese puffer fish) blocks voltage-gated sodium channels, while tetraethylamonium blocks the voltage-gated potassium channel. Both are powerful nerve poisons. General anaesthetics temporarily inhibit the sodium channels. Strychnine blocks glycine receptors in the brain, causing muscle convulsions and death.

2. Drugs acting on the somatic nervous system

Curare and α-bungarotoxin (both snake venoms) block the nicotinic acetylcholine receptors in the somatic nervous system, and so relax skeletal muscle. *Myasthenia gravis* (a weakening of the muscles in the face and throat caused by inactive nicotinic acetylcholine receptors) is treated by the drug neostigmine, which inhibits acetylcholinesterase, so increasing the amount of acetylcholine at the neuromuscular junction. Nerve gas and organophosphate insecticides (DDT) inhibit acetylcholinesterase, so nicotinic acetylcholine receptors are always active, causing muscle spasms and death. Damaged tissues release prostaglandins, which stimulate pain neurones (amongst other things). The non-narcotic analgesics such as aspirin, paracetamol and ibuprofen block prostaglandin production at source of pain, while paracetamol has a similar effect in the brain. Local anaesthetics such as procaine block all sensory and motor synapses at the site of application.

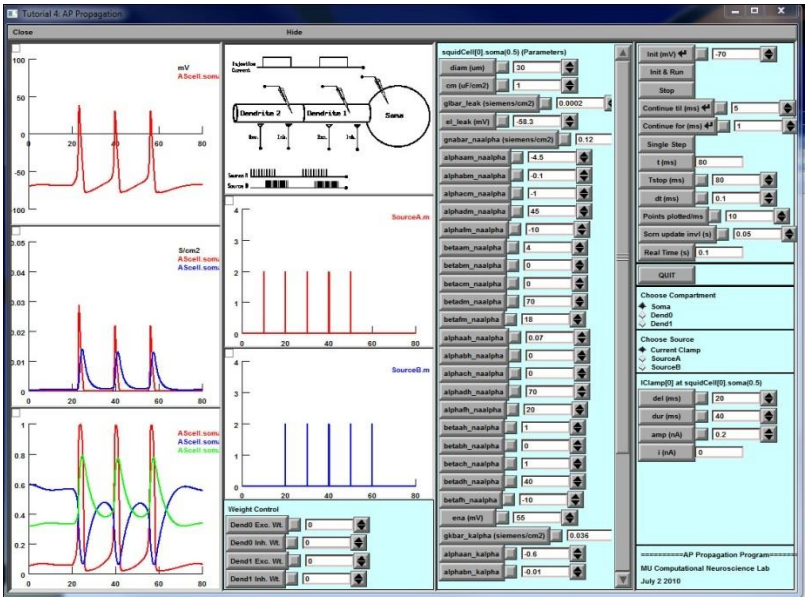
3. Drugs acting on the autonomic nervous system

Sympathetic agonists like salbutamol and isoprenaline, activate the adrenergic receptors in the sympathetic system, encouraging smooth muscle relaxation, and are used as bronchodilators in the treatment of asthma. Sympathetic antagonists like the beta blockers block the noradrenaline receptors in the sympathetic nervous system. They cause dilation of blood vessels in the treatment of high blood pressure and migraines, and reduce heartbeat rate in the treatment of angina and abnormal heart rhythms. Parasympathetic antagonists like atropine (from the deadly nightshade *belladonna*) inhibit the muscarinic acetylcholine receptors in parasympathetic system, and are used as eye drops to relax the ciliary muscles in the eye.

SOFTWARE EXPERIMENT #4a ASSIGNMENT

SYNAPSES Tutorial [adapted from a similar GENESIS tutorial (Bower and Beeman, 2007) by Charlie Franklin and Henry Chen]

Assuming that NEURON is already installed on your systems, go to the folder where you saved the squid tutorial and double click on **SYNAPSES.hoc.** This will open the NEURON software and start the synapse tutorial. You should see a screen that looks something like this.



The SYNAPSES simulation explores the effects of temporal summation for multiple synaptic inputs. Figure 1 shows a schematic of the biological situation in this tutorial, as well as the model. The model is a three compartment model with two dendrite compartments and a soma (which is really more indicative of an axon or spike initiation zone). The model cell receives synaptic input from the equivalent of two pre-synaptic cells: one excitatory, one inhibitory. Each pre-synaptic cell makes a synaptic contact with both dendrite compartments. The Soma is modeled using a Hodgkin-Huxley model for sodium and potassium channels.

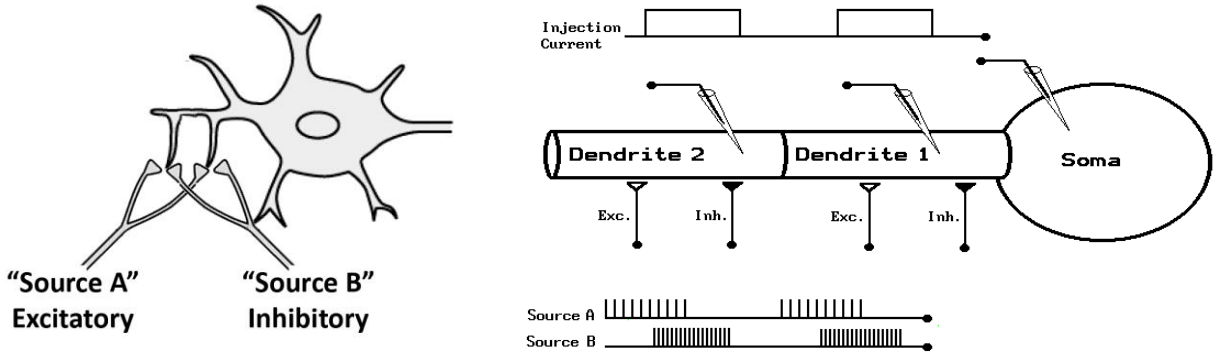


Figure 1 - Neuron Inputs (The figure on the right has been taken from a tutorial in GENESIS by Bower and Beeman, 2000).

The simulation (**S4 SYNAPSES.hoc**) loads with a number of defaults set. You should see five graph windows on your screen. Click on *Init & Run* button to run simulation with default parameters. Check bottom right corner for the default value of Current injection which is set to 0.2 nA. You will notice that synaptic weights for all the sources and dendrites are set to 0. Therefore, only the injection current is currently stimulating the cell.

Note about parameters on the screen:

- Do not change any channel properties, i.e., ignore the third panel
- Do not change anything in the Simulation Control panel (top of 4th panel - InitV, etc.) – just use 'Init&Run' when you want to run the simulation
- 'Choose Compartment to Display' lets you switch between the three compartments displayed in panel 1
- 'Set Source Parameters' can be used to select properties of the input signals. Note: By default, Source A is for excitatory inputs only, and Source B is for inhibitory inputs only.

QUESTIONS – PART A

1: In 20 words or less, what is the relationship between the plot of soma V_m and Dendrite 1 (dend1) V_m? (To switch to Dendrite1 V_m plot just click on *Dend1* button on the right.)

2: Set the Current Injection to 0. This can be done by changing the amplitude of Current clamp to 0. Click on *Init & Run* button to run simulation. You should get a lot of flat lines. Make sure you understand what you've just done.

Leave the soma injection current set to zero. Set the synaptic weight for dendrite1 excitatory input to 12. Note that the "SourceA interval" is set to 10. Click on *Init & Run* button to run simulation. Study what you see. Now change the "SourceA interval" to 3. To display SourceA parameters (which goes to excitatory synapses) just click on SourceA button. Click on *Init & Run* button to run simulation. Repeat this for "Source A intervals" of 2, 1, and 0.5.

In 10 words or less, what principle does the change in Dendrite1 plot indicate? (There is a two-word phrase for this). Also, show a plot for each case.

3: Make a plot of the input-output transfer function. That is, plot output rate vs input spikes rate. For the various cases, make sure that interval*number is a constant at 50 for parameters in the Source A and Source B boxes.

4: Now set the "Source A interval" to "2", “Source A number” to 25, and change the synaptic weight of Dendrite1 Exc. Input to 12. Set the synaptic weight for Dendrite1 Inh. input to 12. Leave everything else as it was. Click on *Init & Run* button to run simulation. You should note that the inhibitory input has little effect upon the generation of action potentials.

Using only the "synaptic weight for Dendrite1 inhibitory input" and the three "Source B" (note: this feeds the inhibitory synapses) parameters (delay, number and interval), inhibit (suppress) the middle of the three action potentials produced by "Source A" input. You may not modify any "Source A" parameters, and both the first and last action potentials must remain. Answer the question by stating the parameter values you had to use. Also, show a plot for every case.

5: Change the weight of Inh. Input for Dendrite1 to 0 and set Inh. Input for Dendrite2 to 10. For this configuration, is the inhibitory synapse more or less effective at suppressing the middle action potential, compared to the one in Question 4? Defend your answer with numbers by varying the synaptic weights (go to the first decimal level) used in this configuration and the previous one. Give reasons for the results which you see. Also, show a plot for every case.

6: Reverse the inputs. That is, place the excitatory input on Dendrite2 (leave the weight at 12) and the inhibitory input on Dendrite1. For this configuration, is the inhibitory synapse more or less effective at suppressing the middle action potential? Defend your answer in the same manner as in QUESTION 5, and explain the differences between this situation and the previous one. Again, show a plot for every case.

Software Experiment #4b RECAPITULATING
THE EARTHWORM ESCAPE REFLEX IN A MODEL – SYNAPTIC SUMMATION

Content areas: Basic neuroscience covered in biology, physiology, psychology, and engineering courses

Pre-requisite knowledge: Cell biology, action potentials, synaptic physiology

Learning Objectives: After this lesson, students should be able to:

- relate a direct "hardware" experiment to a model (earthworm reflex)
- understand the role of synapses in communication between neurons
- understand the role of summation in generating a behavioral response

Time Required:

Keywords chemical synapse, neurotransmitter, pre-synapse, post-synapse, summation

Summary This lesson illustrates the mechanisms underlying the generation of a reflex action in an earthworm. These mechanisms include activation of sensory neurons, which, in turn activate an interneuron, and then the muscle to enable the earthworm to escape. The contrast of summation vs. single-spike mediating transmission becomes apparent.

INTRODUCTION / MOTIVATION

Please refer to the write-ups related to the earthworm experiment for further details about the earthworm biology and its escape reflex.

Weak stimulation of the worm's posterior end elicits one or few action potentials of the LGF. The action potentials have amplitudes in the order of 50 to 200 µV (note that the signal on the screen is multiplied by the amplification factor of the amplifier). The bi-phasic action potential starts with its negative half-wave, indicating that the action potential travels from back to front.

Stronger stimulation of the posterior end leads to more action potentials of the LGF at a faster repetition rate, followed by muscle potentials. Muscle potentials are longer in duration (> 10 ms) and of larger amplitudes (ca. 1 to 10 mV) than action potentials of the giant fibers. After strong stimulation often long 'mountains' of muscle potentials occur, with more LGF action potentials superimposed on them.

Mechanosensory neurons in the skin of the worm's posterior end are connected directly to the LGF. Activity of these sensory neurons (not visible in the recording) causes action potentials of the LGF: the number of action potentials and their frequency depends on the strength of the presynaptic activity (and thus the stimulation). The LGF synapses then with motor neurons which innervate the length muscle. Only several action potentials of the LGF elicit action potentials of the motoneurons. Each action potential of a motor neuron elicits a muscle-contraction, visible as muscle potentials in the recordings. Because of this 1:1 transmission, the synapses between motor neuron to muscle are called 'relay-synapses'.

In contrast to action potentials of the LGF, each MGF is followed by a summed action potential of (giant) motor neurons and a muscle potential. The differences between the LGF and MGF illustrate differences in *synaptic strength* and reveal that LGFs require a summation response to trigger a downstream effect. What are the biological implications of these differences? This means that every AP of the MGF elicits a response (= twitch of the length muscle) so that the more valuable anterior end is better protected than the posterior end.

SOFTWARE EXPERIMENT #4b ASSIGNMENT

Follow the instructions given in 'Software Experiment 4 – How do neurons communicate via synapses' to start the simulation SYNAPSES which we will use for this part also.

Applying the SYNAPSES Model to the Earthworm Experiment

The SYNAPSES simulation explores the effects of temporal summation for multiple synaptic inputs. Figure 1 shows a schematic of the model, which contains spatially separated dendrite compartments, with excitatory and inhibitory synaptically activated channels in both. The soma is modeled using Hodgkin-Huxley equations for Na^+ and K^+ channels.

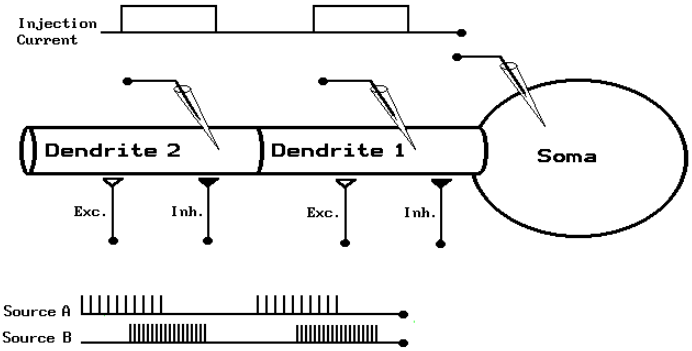


Figure 1 - Inputs to the neuron (figure taken from a tutorial in GENESIS by Bower and Beeman, 2000)).

How can we use this tutorial to simulate the earthworm escape reflex?

For the purposes of this exercise, we will only be using the excitatory inputs. We will assume the neuron pictured above is a motor neuron upon which both LGF and MGF fibers converge. Dendrite1 receives excitatory input from the LGF, Dendrite2 receives excitatory input from MGF. The "soma" in this case is poorly named…we are using it to simulate an action potential being generated in the motor neuron, which of course occurs in the axon.

QUESTIONS – PART B

Stimulation of the Posterior End. Mechanosensory neurons in the worm's skin posterior end are connected directly to the LGF. Activity of these neurons causes action potentials of the LGF, which in turn synapses with the motor neuron, producing movement.
We know that a weak stimulation of the worm's posterior end elicits one or few action potentials of the LGF. A strong stimulation leads to more actions potentials of the LGF at a faster repetition rate, followed by muscle potentials.

1. Sketch the connection between mechanosensory neurons and the LGF and how you think this connection is established in terms of the Neuron simulation model.

2. Now to model this, we need to first conceptualize the following and implement it:

a) Few spikes of the LGF with low frequency (spaced apart from each other) should not produce action potentials in the muscle.

To implement this:

- In the original window, change **Dend1 Exc. Wt** to 10 (this represents the 'strength' of the synapse).
- Change **Soma Inj.** to 0 nA by changing the Current Clamp amplitude to 0.
- Choose **Source A** for your stimulation. Set an **interval** of 10 ms and **width** to 30.
- Press **Init&Run** and see if we succeed in generating an action potential.

b) We saw that biologically 3 action potentials was sufficient to generate an LGF response (see H1-3, Fig 2). So what parameters do we need to change to mirror this biologically?

To implement this:

- Click on SourceA. Change the values for **Interval** (repetition rate) to determine how rapidly the APs must come to trigger a response. Click **Init&Run** for each new value entered and observe your results. This will involve some iteration.

Below what inter-spike interval (note: inverse of this is frequency) was no action potential observed? Above what inter-spike interval did you observe action potentials?

3. Explain the observations in #2 above using neurophysiology and principles of sensory coding.

4. Using the LGF model, explore the relationship between **Interval**, **Number**, and **Dend1 Exc. Wt.** in the generation of a response. Do this by fixing one parameter at a time, and explore the relationship between the other two. Make a table listing all your values systematically and draw conclusions.

Stimulation of the anterior end. Differently from the spikes of the LGF, each MGF spike is followed by a summed action potential of the motor neuron and a muscle potential. This means that every spike of the MGF produces a response in the earthworm's muscle. During anterior end stimulation, the mechanosensory neurons are excited. They are connected to interneurons through excitatory synapses, which in turn excite the MGF. The MGF then has excitatory connections with the motor neurons.

5. Change the values for **Interval** (repetition rate) and **number** in order to obtain the scenario above. Describe your methodology and findings.