Neurophysiology

V. Neurotransmitters and the Autonomic Nervous System:
A Modified Loewi’s Experiment

Materials

Latex gloves
Bull Frog
Dissecting tools
MacLab® Data Acquisition Equipment and Chart software
Ringer syringe with 26 gauge needle
0.05M epinephrine syringe with 26 gauge needle
0.01M acetylcholine syringe with 26 gauge needle
Acetycholine and atropine sulfate syringe with 26 gauge needle

Purpose

To investigate the effects of the sympathetic and parasympathetic divisions of the autonomic nervous system on the heart by exogenously administering different neurotransmitters. To pharmacologically demonstrate the effects of an antagonist on a neurotransmitter system.

I. Introduction

Intercellular Chemical Transmission

In 1920 Otto von Loewi provided the first empirical evidence that chemical substances are responsible for the bulk of communication between individual neurons and neurons and their target organs (Appendix A is an excerpt from Loewi’s own notes about his revolutionizing discovery). Scientists have since discovered, named, and identified the effects of many of these endogenous (internally produced) substances. Methods have also been developed that permit efficient purification and/or synthesis of these substances so that scientists can purchase these exact chemicals through distributors (much like Christmas shopping from the Eddie Bauer catalog), and administer them to biological preparations under very precise conditions. We will be taking advantage of the exogenous application of two neurotransmitters, acetylcholine and epinephrine, to study their effects on the vertebrate heart. The reason we will use these two specific transmitters will become apparent momentarily, until then, we need to understand some features of cardiac anatomy and physiology.

2 Contributed by Kurt D. MacDonald, Fall 1997
The Vertebrate Heart

The vertebrate heart primarily consists of cardiac muscle. This muscle, like ordinary muscle, conducts impulses, and it is this electrical activity that signals the heart to contract. While you might expect that this electrical signal is generated in the central nervous system, it is actually initiated by a specialized cardiac structure called the sinoatrial (SA) node. The SA node, located in the wall of the right atrium just below the superior vena cava (Fig. 1), is composed of a bundle of specialized cardiac cells that are inherently rhythmic. That is, even if all neural input to this area is eliminated, it will continue to initiate impulses at regular intervals. Furthermore, because the SA node’s inherent rate of depolarization is greater than that of ordinary cardiac muscle, its activity initiates mechanical contraction of the entire heart. Because of this property, the SA node is sometimes called the heart’s pacemaker.

Each time an impulse is generated in the SA node, it spreads through the muscle fibers of both atria exactly like an action potential travels down the length of an axon. The presence of electrotonic synapses (gap junctions) between all cardiac muscle cells ensures that this conduction is both exceedingly fast and reliable (so your heart never misses a beat).

As the stimulated atria are contracting, the action potential enters another specialized cardiac structure in the right atrium: the atrioventricular (AV) node (Fig. 1). As the action potential enters the AV node its conduction slows markedly. This delay permits the atria to fully contract before the impulse initiates ventricular contraction. Thus, the AV node is responsible for establishing the sequence between atrial and ventricular contractions. Once the electrical impulse has passed through
the AV node, it is widely distributed to the ventricles by way of an elaborate conduction network comprised of the AV bundle and Purkinje fibers (Fig. 1).

From the information just presented, it would be logical to assume that normal heart functioning does not require any neural input. It is true that the heart is myogenic (generates its own inherent rhythm), however it also receives rich innervation from the sympathetic and parasympathetic divisions of the autonomic nervous system (Fig. 2). It is this input that adjusts the heart’s internal rhythm.

**FIGURE 2**

Sympathetic control of the heart originates in the cardioaccelerator center of the medulla and reaches the heart by way of sympathetic fibers in the middle, superior, and inferior cardiac nerves. When sympathetic pathways are stimulated they release the neurotransmitter norepinephrine from their long postganglionic cells. When norepinephrine is released from sympathetic terminals that innervate the heart, it primarily activates β-adrenergic receptors. These receptors influence the heart in a variety of ways. When the β-adrenergic receptors of the heart are active they increase the heart muscle’s Ca++ current. This causes an increase in the heart’s force of contraction. Additionally, β-adrenergic
activation causes depolarization of the SA node. This, in turn, causes the heart to beat faster. The effects of norepinephrine on the heart can be seen in figure 3.

Parasympathetic impulses from the cardioinhibitory center in the medulla decrease heart rate and cardiac contractility. This inhibitory action is mediated by the release of acetylcholine from the vagus nerve (cranial nerve X). Free acetylcholine acts on muscarinic receptors which hyperpolarize the cells of the SA node and slow the conduction of the action potential through the AV node. This slows heart rate. Acetylcholine also decreases Ca\(^{++}\) influx which lowers the heart’s force of contraction. The effects of vagal nerve stimulation, and hence acetylcholine release, can be seen in figure 4.

At any given time, both divisions of the autonomic nervous system are influencing heart function. However, in a normal resting heart (you sitting in your Lazy-boy reading this lab) the influence of the parasympathetic division is greatest. Thus, when the heart is at rest, we say that the predominant tone is parasympathetic. It is only with additional sympathetic activation, the so-called fight-or-flight response (you frantically reading this lab 1 minute before your quiz) that the effects of the sympathetic division are paramount.

In this experiment you will use the MacLab hardware and software to monitor the heart’s response to injections of different chemical substances. This recording will allow you determine heart rate and force of contraction. In the first condition you will administer a Ringer solution (a biologically inert solution) and quantify the heart’s response. In the second condition you will administer an injection of 0.05M epinephrine while monitoring heart function. The third condition involves applying 0.01M acetylcholine to the heart. In the final condition you will apply a mixed solution of acetylcholine and the cholinergic antagonist atropine sulfate.
II. Experimental Setup

Some of the data acquisition hardware you will use in this experiment is identical to the equipment used in the introductory dipole experiment. It consists of the MacLab/400 unit, ETH-250 bridge/bioamplifier, and the Macintosh PowerPC. A new component of hardware is the displacement transducer. As its name implies, this apparatus transforms physical displacement (movement) into an electrical signal that the computer can read.

The data acquisition software required for this lab is also new. In this lab you will be using MacLab’s Chart program. This software emulates an old fashioned chart recorder—an apparatus that records changes in a preparation over time by moving a pen up and down while paper is fed through the machine at a constant speed. (You may be most familiar with the use of chart recorders in the field of geophysics—they are the machines often shown wildly responding to the tremors produced by earthquakes).

III. Method

Because of the myogenic property of the heart, we can perform this experiment on a dead frog. However, the frog must be recently deceased for the experiment to be successful. Your TA will perform a humane method of euthanasia; the rest of the experiment is up to you.

Heart Exposure

We will be performing this study in vivo (within the body), so you will need to CAREFULLY expose the beating heart.

1) With the frog lying on its back (ventral-side up), use a sharp scalpel to make a small opening in the tough outer skin near the rostral boundary of the sternum. Place a tip of the scissors into this opening and make a longitudinal incision that extends along midline to the level of the lower-abdomen. This cut should only pass through the outer skin.

2) Using the scissors, make two perpendicular cuts (one at each end of the initial cut) that will permit you to fold the outer skin away from the midline as two separate flaps (very fine use of the scalpel may be necessary to separate the tough outer skin from the underlying tissue). Examine the beautiful arterial system present on the underside of these flaps.

3) The frog heart, similar to our mammalian heart, is located under the sternum and is protected by a ribcage. To approach the heart, we will begin by making a small incision in the wall of the abdomen at the level of the frog’s imaginary navel (frogs don’t have umbilical cords, hence they have no navels). Extreme care must be taken to avoid puncturing the delicate tissue and organs that underlie the abdominal musculature.

4) Using scissors, extend this cut in the rostral direction until you approach the ribcage (do not damage the scissors by attempting to cut bone). Near the rostral end of this cut, near the base of the rib cage, make two transverse/horizontal cuts in the abdominal wall (if you are confused where to make this cut, ASK your TA). At this point you should have a clear view of the frog’s liver, stomach, intestines, and egg sac (if it is female). You should also be able to see the beating heart if you gently life the sternum and look under the ribcage.

5) After locating the heart, and ensuring yourself that you will not damage it, use the bone snips to cut up both sides of the rib cage. Separate these cuts with sufficient distance so that you will have ample space around the heart in which to work.
6) Remove the flap created in step #5. The myogenic heart, encased in an opaque membrane (pericardial sac), should be in clear view! (You can use a wooden stick to spread the chest cavity open so that you have a larger working area around the heart).
7) The final step involves carefully cutting away the pericardial sac (do not cut any of the heart’s entering or exiting vasculature).

Setting up the MacLab Hardware and Software

1) Make sure that the displacement transducer is plugged into Channel 1 on the back of the ETH-250 amplifier, and that the output of the ETH-250 is connected to the CH1 input of the MacLab unit.
2) Set the Mode to bridge, Gain to 100, and Filter to W.B. (wideband).
3) Double click on the Chart icon in the MacLab folder. After some initialization, the main chart window is shown. By default, Chart displays 8 channels. To the right of the display window for a given channel are some controls for that channel. To the right of channel 2, click and hold on the Channel 2 control and select “Turn channel off...” from the pop-up menu. Repeat this process for channels 3 & 4 (channels 5-8 are off by default).
4) Click and hold on the double line separating the display window for channel 1 and channel 2. Drag this line to the bottom of the screen so that the display for channel 1 occupies the entire screen.
5) Choose Display settings from the Setup menu and click the Always seconds box in the dialog window that appears.
6) To the right of the Channel 1 window, click and hold on the Channel 1 control, and select Input Amplifier from the pop up menu. Make sure that both the Positive and Negative boxes are selected. Gently tap the displacement transducer and note that a response is generated on the display.
7) Decrease the Range to 50 mV and adjust the Input Offset knob on the ETH-250 to center the signal in the display. (Throughout the experiment, there may be times when the display reads out of range. When this occurs, leave the Range at 50mV and adjust the Input Offset to return the signal to within the visible range). After you have done this, click OK.
8) In the main chart window, click on Start in the lower right corner. The display begins to scroll, recording the movement of the displacement transducer. Note that elapsed time (in seconds) is displayed above the Channel 1 control. You may change the sweep rate (i.e. how fast the “paper” is moving in the window) by clicking and holding the arrow box next to the elapsed time.

Linking the Heart to the Displacement Transducer

To accurately quantify changes in heart rate and force of contraction you will be using the displacement transducer. To properly attach the heart to the displacement transducer, use a threaded suture needle to tie the tip of the ventricle to the end of the displacement transducer (don’t worry about the string tension when you are tying the knot, you can adjust the tension by changing the location of the transducer on the ringstand). Raise the transducer on the ringstand to slightly elevate the heart and take the slake out of the suture thread. A signal should be displayed on the chart display.
IV: Experimental Conditions and Data Worksheet

<table>
<thead>
<tr>
<th>Condition</th>
<th>Delay (Sec.)</th>
<th>Latency of the last peak on the screen (sec)</th>
<th>Latency of the first peak on the screen (sec)</th>
<th>Number of peaks on the screen</th>
<th>Heart Rate</th>
<th>Change in rate from previous condition</th>
<th>Amplitude of the first peak on the screen</th>
<th>Amplitude of the last peak on the screen</th>
<th>Average amplitude of the first and last peak</th>
<th>Change in amplitude from the previous condition</th>
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<tbody>
<tr>
<td>Baseline (no injection)</td>
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<td>0.05ml Ringers</td>
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<td>0.05ml Acetylcholine (0.01M)</td>
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<tr>
<td>0.05ml Acetylcholine and Atropine Sulfate</td>
<td>100</td>
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<tr>
<td>0.05ml Epinephrine (0.05M)</td>
<td>100</td>
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Changing the Experimental Condition

Begin by pressing **Start** and recording a few minutes of baseline heart function. To quantify heart rate and force of contraction, press the **Stop** button in the lower left corner of the display and make the measurements necessary to complete the appropriate section in the following data worksheet (actual instructions on the measurement procedure follow the data worksheet page). Once these measurements are complete, make an injection and immediately press start (notice that the elapsed time counter has reset; the display may also report that the data is **Out of Range**. If this is the case, don’t panic and do not press **Stop**. You may readjust the **Input Offset** while recordings are being made). Complete this series of starts and stops until the data worksheet is complete.

Calculating Heart Rate and Force of Contraction

The data sheet presented on the preceding page is designed to help you complete the calculations necessary to quantify heart rate and force of contraction.

- To record the latency of a peak, place the cursor on the positive point of the peak and read the value (in seconds) from the window above the **Channel 1** control box.
  - one helpful tool that allows you to make far more precise measurements is the blowup window. Ask your TA how to locate this window.
- To calculate heart rate, divide the number of peaks by the time elapsed between the first and last peak and then multiply the result by 60. This will yield the number of heart beats per minute.
- To calculate the change in rate, subtract the old rate from the new rate and divide the result by the old rate. Multiply this number by 100 to get the percent change.
- To measure the amplitude of a single peak, place the cursor on the tip of its most positive peak and record its amplitude from the window above the **Channel 1** control box; then place the cursor on the tip of its most negative peak and record its amplitude. The total height of a peak is calculated by adding the absolute value of its most positive and negative peak. Again, the blowup window will be very useful here.
- To calculate the average amplitude of the first and last peak, add their amplitudes together and divide by 2.
- To calculate the change in amplitude, subtract the old amplitude from the new amplitude and divide the result by the old amplitude. Multiply this number by 100 to get the percent change.

V: Lab Report

Your lab report should follow the introduction-methods-results-discussion format introduced previously.

The introduction should be comprised of general information about the sympathetic and parasympathetic divisions of the autonomic nervous system and how these different divisions innervate the heart (i.e. how these fibers get to the heart). In addition, knowing the neurotransmitters used by the postganglionic cells of the parasympathetic and sympathetic divisions, you should hypothesize what effects these systems will have on the heart when they are increasingly activated. Here it would be appropriate to cite references to support your hypothesis (general neuroscience texts are acceptable).

The methods should be relatively straight forward. You need to describe the experimental setup, including general information about the hardware and software. You also
need to describe each of the experimental conditions in sufficient detail that a reader unfamiliar with the experiment could replicate it on his/her own. This, of course, means including quantities, concentrations, delays, and measurement techniques. Remember, no results should be included in the methods section.

The results section should include 2 tables. The first table should include the data from the 5 columns of the data worksheet that pertain to the heart’s rate (latencies, # of peaks, heart rate, and change in heart rate). The second table should contain all of the data from the remaining columns that quantifies the heart’s force of contraction (amplitudes, average amplitude, change in amplitude). In addition to these two properly labeled tables, include a few brief paragraphs describing the results (be sure to refer to your tables in your text).

The discussion section should compare your results with the results you had expected to obtain. Any discrepancies should be mentioned, and you should theorize why they occurred. Additionally, any unusual results should be explained in this section. (Hint: if you know anything about the mechanism of action of war gasses, now would be a good time to compare that to the results of this experiment.)

References


The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at six o’clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o’clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. I have to describe briefly this experiment since its results became the foundation of the theory of chemical transmission of the nervous impulse.

The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beat diminished just as if its vagus had been stimulated. Similarly, when the accelerator nerve was stimulated and the Ringer from this transferred, the second heart speeded up and its beat increased. These results unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances which, in turn, cause the well-known modifications of the function of the heart characteristic of the stimulation of its nerves.

(Taken from Kandell & Schwartz, 1991)