

Experiment 12: Uterine Motility

Background

Smooth muscle is composed of fibers, which at 2 to 5 microns in diameter and 50 to 200 microns in length, are smaller than those found in skeletal muscle. Although the physical arrangement of smooth and skeletal muscles differ, the same chemical substances are responsible for the contractions of both of these muscle types. Smooth muscle fibers found in different organs are distinctly different from each other in their physical dimensions, organization into bundles or sheets, response to stimuli, characteristics of innervation, and function.

The rat uterus, that will be used in this experiment, is composed of many spindle-shaped cells with small diameters. These cells are electrically coupled to each other at multiple points known as gap junctions. Ions flow freely from one cell to the next through these gap junctions, so that fibers form a functional syncytium (a large area of muscle which contracts in unison). For this reason, the rat uterus is classified as a “single unit muscle.” When a portion of the muscle is stimulated, the action potential is easily conducted to the surrounding fibers by direct electrical conduction; it is as if cell membranes did not exist. Smooth muscles do not have synaptic junctions, like skeletal muscle does. Transmitters like norepinephrine and Acetylcholine are secreted by axons from sympathetic and parasympathetic postganglionic cells, respectively. The chemicals diffuse to the muscle, where they usually modulate existing contractions, which may be endogenously generated or produced by muscle stretch.

Single-unit smooth muscle, found in most organs of the body (gut, bile duct, ureters, uterus) is controlled mainly by non-nervous stimuli (hormones and local factors such as $[O_2]$, $[CO_2]$, and $[H^+]$). As you will see this type of smooth muscle exhibits spontaneous, rhythmic contractions. Smooth muscle can maintain a state of long-term, steady contraction called tonus. This is an important feature which allows prolonged or indefinite continuance of smooth muscle function. An example of tonus would be the tonic contractions of blood vessels throughout the entire life of a person. These contractions result from prolonged direct smooth muscle excitation by local factors or circulating hormones such as angiotensin, vasopressin, or norepinephrine. Smooth muscle can also shorten by a greater percentage of its length than skeletal muscle can: 50 to 75% for smooth muscle vs. 25 to 35% for

skeletal muscle. This characteristic allows the hollow viscera (gut, bladder, blood vessels) to change lumen diameters from zero to very large values.

Another characteristic of smooth muscle is its ability to change length greatly without marked changes in tension. This is known as plasticity and occurs because of a phenomenon called stress-relaxation. Stress-relaxation results from the loose arrangement of the actin and myosin filaments in smooth muscle. The filaments of a stretched muscle rearrange their bonds, causing sliding between the filaments. Within a few minutes, tension returns to its previous level. The converse effect occurs when smooth muscle is shortened. All tension is lost when the muscle length is reduced, but tension gradually returns over a period of one minute or more.

The purpose of this experiment is to demonstrate some of the contractile properties of smooth muscle using an isolated rat uterus: spontaneous contractile activity, the effect of stretching the muscle, and the effects of various agonists on the frequency and the degree of contraction.

Equipment Required

PC Computer
iWorx data acquisition (A/D) unit
USB or serial cable for connecting A/D to computer
FT-302 Force transducer for 0-10 gram and 0-100 gram ranges
STB-125 Student tissue bath
Table-top water bath at 37°C
Heating circulator, set at 37° C (Optional)
Suture thread and needle
Pasteur pipets and bulbs
Air-tight chamber, and dry ice or CO₂ supply
Dissection pan and instruments
Cylinder with a mixture of 95% O₂ & 5% CO₂
Regulator, valve, and tubing for oxygenation setup
Tyrode's Physiological Saline (See appendix)
Various reagents in Tyrode's Physiological Saline (See appendix)

Precautions

- 1 Keep the uterus in well-oxygenated buffer at the experimental temperature at all times. This helps the uterus to function normally for the whole lab period.

- 2 Complete all the lab exercises before taking time to analyze any of the data. The functionality of the uterus is limited by time. Completing the exercises quickly improves the chances of completing the experiment with the same uterus.
- 3 The temperature of the fresh buffer used to rinse the uterus and replace the buffer in the chamber should be the same as the temperature of the uterus. Keep flasks of fresh buffer in the water bath at the same temperature as the uterus and the buffer in the chamber.
- 4 Start the experiment as quickly as possible after the isolation of the uterus. Designate members of the lab group to perform different parts of the equipment setup: opening and setting up the LabScribe software; assembling the tissue chamber, calibrating the transducer; and so on.

Equipment Setup

Assemble the Recording System

- 1 Connect the iWorx A/D unit to the computer with the appropriate USB or serial cable (described in Chapter 1).
- 2 Insert one of the connectors of the DIN8 cable into the socket on the FT-302 dual-range force transducer. Insert the connector on the other end of the DIN8 cable into the input of Channel 3 on the iWorx A/D unit.
- 3 Turn on the iWorx A/D unit and the computer. Make sure these units have been turned on for at least ten minutes before the transducer is calibrated.

Start the Software

- 1 Click the **Windows Start** menu, move the cursor to **Programs** and then to the **iWorx** folder and select **LabScribe**; or click on the **LabScribe** icon on the Desktop
- 2 When the program opens, select **Load Group** from the **Settings** menu.
- 3 When the dialog box appears, select **IPLMV3.iws**. Click **Load**.
- 4 Click on the **Settings** menu again and select the **Intestinal Motility** settings file.
- 5 After a short time, **LabScribe** will appear on the computer screen as configured by the **Intestinal Motility** setting.

Assemble the Tissue Bath

- 1 Assemble the tissue bath. The components of the STB-125 student tissue bath are listed in Table 4-1 on page 2. The completed tissue bath is pictured in Figure 4-1 on page 2.
- 2 Thread the 50cm stainless steel rod (502191) into the matching threaded hole on the back of the white non-magnetic base (502190). With rod in this position, the base will provide a stable support for the components aligned above it.
- 3 Place a plastic parallel frame clamp (502193) on the

stainless steel rod. This clamp should be about 20cm above the surface of the base and aligned as seen in Figure 4-1 on page 2.

- 4 Place the arm of the glassware extension clamp (14016) in the clamping hole on the side of the parallel frame clamp. Position the extension clamp so its prongs can hold the tissue chamber vertically over the front half of the base.

Table 4-1: Components of the STB-125 Student Tissue Bath Needed for this Experiment

Part Number	Part Description
502190	White Non-Magnetic Base
502191	50cm Stainless Steel Rod
47024	25ml Tissue Bath
502193	Plastic Parallel Frame Clamp
14016	Glassware Extension Clamp
4731	Nylon Tubing, 0.153"OD X 0.106"ID
14018	Oxygen Connector
4983	Silicone Tubing, 0.250"ID X 0.438"OD
7465	Polypropylene Pinch Clamp
160172	Glass Tissue Support
502198	Transducer Positioner

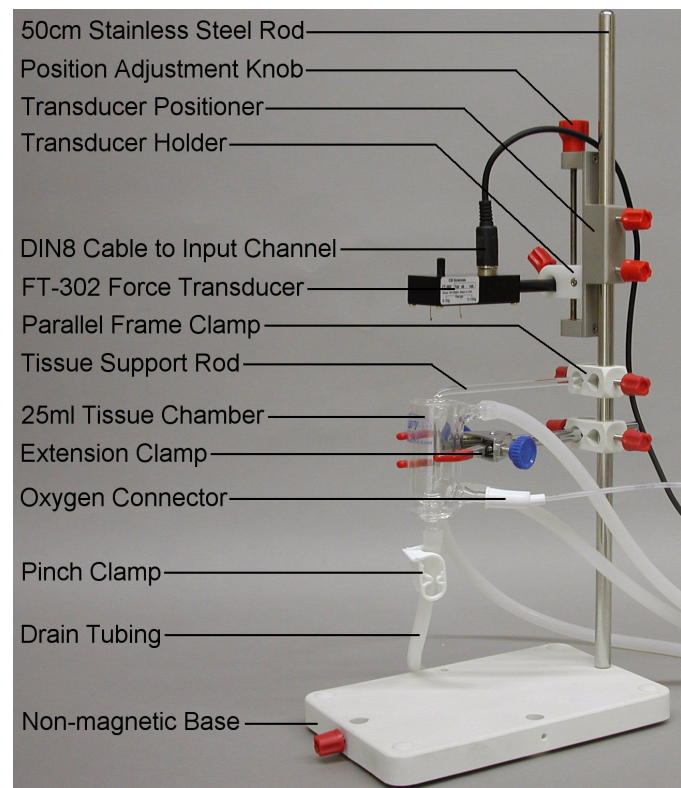


Figure 4-1: STB-125 student tissue bath with a FT-302 force transducer.

- 5 Place a second parallel frame clamp (502193) on the stainless steel rod. Align this clamp above the first clamp. The second clamp will eventually hold the glass tissue support (160172) that will hold the lower end of the uterus in the chamber.
- 6 If you need to hold another device, like an electrode, on the uterus or above the tissue chamber, place the third parallel frame clamp (502193) on the stainless steel rod. The third parallel frame will eventually be a few centimeters above the second parallel frame clamp.
- 7 Place the transducer positioner (502198) on the stainless rod with red adjustment knob of the positioner on top. Clamp the positioner on the rod so the bottom of the positioner is about 30cm above the base.
- 8 Clamp the FT-302 dual-range force transducer in the holder on the threaded rod of the positioner (Figure 4-1 on page 2). Align the positioner and the transducer so the hook on the transducer is directly over the center of the tissue chamber. Use the hook for the appropriate range of tension you expect in the experiment.
- 9 Turn the red adjustment knob on the positioner to move the transducer and its holder to the middle of the threaded rod.
- 10 Determine the best location on the lab bench for the tissue bath setup. The setup should be convenient to a sink or a drain flask, the mixed gas supply used for aeration, a water bath used for warming flasks of buffer used in the experiment, a warm water supply or a heating circulator used to maintain the temperature of the uterus, and the data acquisition system used to record the responses of the uterus during the experiment.
- 11 Cut the silicone tubing into the lengths needed used to make a drain line, an overflow line or a supply line to a buffer reservoir, and water lines to and from a warm water supply needed to maintain the temperature of the tissue bath.
- 12 Feed about 8cm of the drain line through the two circular holes on the plastic pinch clamp (7465). Carefully put the end of the drain line with the pinch clamp on the drain port of the tissue bath (Figure 4-2 on page 3). Leave the drain open.
- 13 Carefully put the ends of the additional fluid lines on the appropriate ports of the tissue bath (Figure 4-2 on page 3).
- 14 Put the oxygen connector (14018) on the aeration port of the tissue bath.
- 15 Carefully clamp the assembled tissue bath between the prongs of the glassware extension clamp already on the stand.
- 16 If the tubing needed on the tissue chamber has not been prepared, find the coils of silicone (4983) and nylon (4731) tubing.
- 17 Attach one end of the nylon tubing (4731) to the oxygen connector on the tissue chamber. Connect the other end of the nylon tubing to the valve on the cylinder containing 95% O₂ and 5% CO₂.

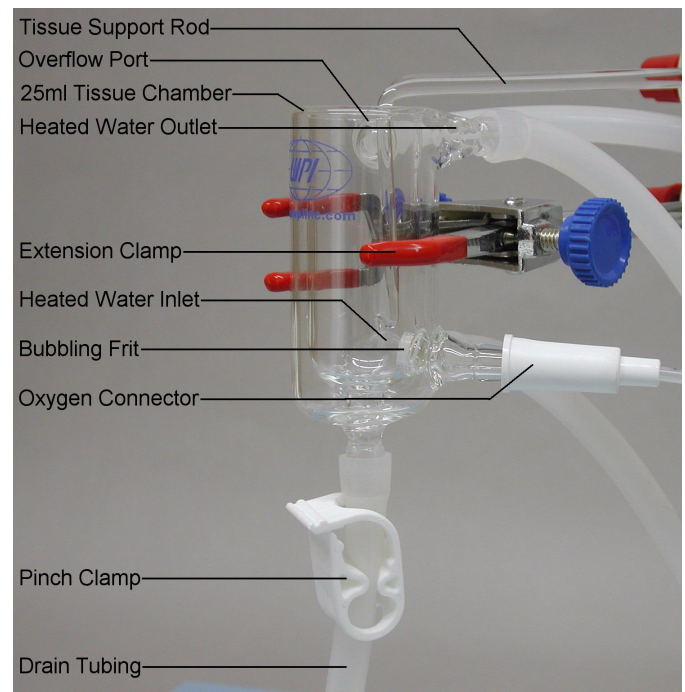


Figure 4-2: 25 ml tissue bath in position for recording tissue tension.

- 18 Place the end of the drain line in the sink or a flask used to collect waste buffer. Connect the tubing on the water inlet, at the bottom of the tissue bath, to the warm water supply at the sink or to the outlet of a heating circulator. Place the end of the tubing on the water outlet, at the top of the tissue bath, in the sink or on the inlet of the heating circulator.

Calibrate the Transducer

- 1 Make sure that the iWorx unit is turned on and the transducer is connected to the input for ten minutes before the calibration is done.
- 2 Before attaching the uterus to the transducer, click **Start** and type "No Weight" on the comment line to the right of the **Mark** button. Press the **Enter** on the keyboard. Record a baseline with no weight hanging from the arm or hook of the transducer.
- 3 While recording, type "5 grams" on the comment line to the right of the **Mark** button. Hang a 5 gram weight on the arm or hook of the transducer. Press the **Enter** on the keyboard. Record for 10 more seconds. Click **Stop**.
- 4 Click the **2-Cursor** icon (Figure 4-4 on page 5). Two blue vertical lines appear over the **Main** window. Move one cursor to the baseline of the recording when no weight was hanging on the transducer. Move the second cursor to the plateau where 5 grams of weight was hanging on the transducer.
- 5 Open the **right-click** menu and select **Units**. Enter "0" (zero) in the box across from the amplitude at the first cursor. Enter "5" in the box across from the amplitude at the second cursor. Enter the **Name** of the units as "grams."

Prepare the Tissue

The uterus should be dissected from an adult female rat as follows:

- 1 Sacrifice the rat by placing it in the air-tight chamber with a piece of dry ice. Carbon dioxide is emitted as the dry ice warms quickly; this humanely kills the rat. Place the rat on its back in the dissection pan and make a mid-line incision along the lower half of the abdomen.
- 2 Displace the intestines to one side to expose the two "horns" of the uterus (Figure 4-3 on page 4).
- 3 Tie a suture (15cm long) around the anterior end of each horn of the uterus. Carefully remove any fat and mesentery from the uterus. Tie another suture around each horn close to the point where the uterus bifurcates into the two horns.
- 4 Remove each horn from the rat. Avoid stretching the uterus. Place both horns in a beaker of aerating physiological saline at 37°C.

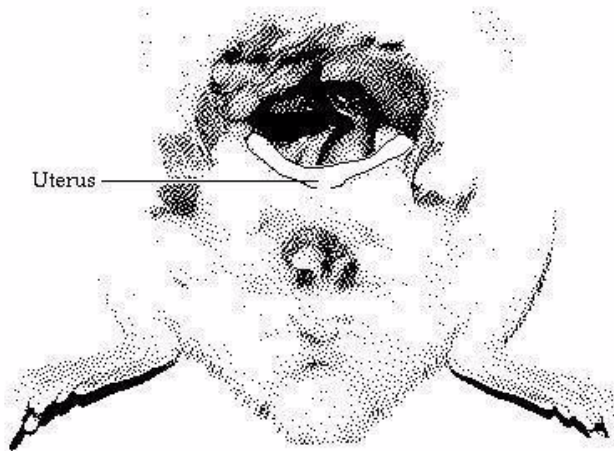


Figure 4-3: Diagram showing the uterine horns in the lower abdomen of a female rat.

Place the Tissue in the Chamber

- 1 Use a clean beaker to obtain about 100 ml of Tyrode's saline at 38°C from one of the large flasks in a water bath. Take only as much Tyrode's as you need for each rinse or buffer change. Reserve this beaker for transferring clean Tyrode's throughout the exercise.

Note: Avoid contamination! Do not return any Tyrode's solution taken from the supply flask back to the supply flask!

- 2 Rinse the tissue chamber thoroughly, three or four times, with Tyrode's solution.
- 3 Close the drain of the tissue chamber and fill the chamber with about 20ml of Tyrode's solution. Open the valve on the aeration line and adjust the flow of the oxygen/carbon

dioxide mixture through the aeration frit to create a plume of small bubbles.

- 4 Obtain a uterine horn to use in the experiment. Keep the uterus in a beaker or dish of buffer at the desired temperature until you are ready to attach it to the support rod.
- 5 Work quickly and carefully when mounting the uterus in the chamber. Attach the one end of the uterus to the glass tissue support using a loop of suture thread securely tied to the end of the uterus and looped under the hook of the tissue support rod. Securely tie a piece of suture to the other end of the uterus. Make sure the suture is long enough to connect the uterus to the transducer. Tissue clips (501902, 501903) can also be used to attach the uterus to suture threads on the hooks of the tissue support and transducer. Clips may slip off the uterus if the force developed by the uterus is greater than the grip strength of the clips.
- 6 Once the lower end of the uterus is attached to the hook of the tissue support rod (160172), lower the uterus and its support rod into the tissue chamber. Keep tension on the upper suture thread as the assembly is lowered into the chamber. This will prevent the uterus from coming off the hook on the support rod.
- 7 Attach the suture thread on the upper end of the uterus to the appropriate hook on the arm of the transducer. The length of the uterus should be no greater than its *in situ* length.
- 8 Align the transducer, the tissue bath, and the tissue support rod. The suture and the uterus should be vertical, and the uterus should not be touching the inside of the tissue bath.
- 9 Check the temperature of the tissue bath. Designate a member of your lab group to monitor the temperatures of the tissue bath and water bath holding the flasks of fresh buffer. It will take five to ten minutes for the uterus to recover normal function after it is placed in the warm tissue bath. Slow waves of contraction through the horn should be clearly visible once normal function has been restored.
- 10 Start recording the tension in the uterus. Click on the **Start** button in the upper right corner of the **LabScribe Main** window. Click the **AutoScale** button on the upper margin of the recording channel. Observe the position of the trace on the screen as you gradually raise the transducer by turning the adjustment knob on the positioner. Turn the knob until the trace on the screen visibly moves from its initial level. The amount of adjustment required depends on the initial slack in the uterus and the threads holding the uterus.
- 11 If necessary, adjust the flow of bubbles from the aeration frit to prevent the uterus from being moved around by the bubbles.

Note: If contractions in the tissue are visible, but do not produce a noticeable movement in the recording, check the tension of the suture threads holding the tissue in place and the operation of the transducer and the recording system.

Exercise 1: Spontaneous Contractile Activity

Aim: To measure the frequency and amplitude of spontaneous contractions in the rat uterus.

Procedure

- 1 Click **Start**. Type “Normal” on the comment line to the right of the **Mark** button.
- 2 Press the **Enter** key on the keyboard. Record the uterine muscle activity. Record until the contraction cycles are consistent and predictable. It may take as long as 30 minutes for the uterus to return to a consistent rhythm after it has been isolated from the rat.
- 3 **Stop** to halt recording.
- 4 Select **Save As** in the **File** menu, type a name for the file. Choose a destination on the computer in which to save the file (e.g. the **iWorx** or class folder). Click the **Save** button to save the file (as an *.iwd file).

Data Analysis

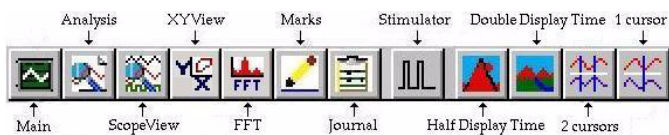


Figure 4-4: The LabScribe toolbar.

- 1 Use the **Double Display Time** icon in the LabScribe toolbar to adjust the time so that two uterine contraction cycles are displayed on the **Main** window. Click **AutoScale** to maximize the size of the response on the window.
- 2 Click the **2-Cursor** icon (Figure 4-4 on page 5), so that two blue vertical lines appear over the recording window. Move the cursors to positions on the recording window to measure the following parameters:
 - The amplitude of the active contraction, which is **V2-V1**, the voltage difference between the baseline and peak of the contraction. This active contraction is known as the phasic response.
 - The period of the contraction, which is time difference (**T2-T1**) between the peaks of adjacent cycles.
 - The frequency of contraction, which is the inverse of the period.
 - The level of the baseline before each contraction cycle. The position of the baseline is a relative measure of the passive tension, or tone, of the resting uterine muscle.
- 3 Data can be entered in the **Journal**, by clicking on the **Journal** icon in the LabScribe toolbar, and typing the measurements into the **Journal**.
- 4 Measure values for two additional cycles adjacent to the first cycle examined. Calculate and record the means for each parameter.

Exercise 2: Effects of Various Agonists

Aim: To examine the effects of different concentrations of drugs on contraction amplitude and frequency.

The following drugs will be used: oxytocin; acetylcholine; atropine (blocks choline receptors), followed by a drop of the acetylcholine solution; epinephrine. Other drugs, that are optional, include: leucine enkephalin, an endogenous opiate with morphine-like effects; naloxone (reverses the effects of opioid drugs), used while leucine enkephalin is still in the chamber; methergine (ergot alkaloids), commonly used as an obstetrical herb to increase frequency and force of contraction.

Procedure

- 1 Click **Start**. Type “Control” on the comment line. Press the **Enter** key on the keyboard, and record a couple of consistent spontaneous contractions of the uterus.

Note: Administer the drugs to the prep in the following order: 1. Oxytocin; 2. Leu Enkephalin; 3. Naloxone; 4. Methergine; 5. Acetylcholine; 6. Atropine, then Acetylcholine; 7. Epinephrine. Drugs 2, 3, and 4 are optional.

- 2 While recording, type the “Oxytocin” on the comment line to the right of the **Mark** button.
- 3 Add the prescribed amount of Oxytocin to the muscle chamber. Press the **Enter** key on the keyboard to mark the recording at the same time the drug is added to the chamber.
- 4 Click **Stop** when the response appears consistent.
- 5 Select **Save** in the **File** menu.
- 6 Remove the bath fluid containing the drug from the muscle chamber. Rinse the uterus preparation carefully, with fresh physiological saline at 37°C. Rinse the prep a second time. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
- 7 Rinse the muscle chamber with fresh physiological saline, twice. Refill the chamber with fresh physiological saline at 37°C.
- 8 Click **Start**. Type “New Normal” on the comment line. As the preparation equilibrates, record the spontaneous activity in the uterine muscle. When the contractions are consistent, press the **Enter** key.
- 9 Type the name of the new drug on the comment line, and press the **Enter** key as the dose of drug is added to the muscle chamber. Click **Stop** when the response appears consistent. Select **Save** in the **File** menu.
- 10 Repeat Steps 6 through 9 for each new drug.
- 11 Remember to rinse the last drug from the prep and the chamber, and refill the chamber with fresh physiological saline at 37°C.

Data Analysis

- 1 Scroll to the appropriate section of data for each drug. Use the cursors to measure tone, contraction amplitude, period, and frequency.
- 2 Enter the data in the **Journal** and construct a table to display these parameters for the controls and each of the drugs.

Questions

- 1 For each drug:
 - What is the effect of the drug on the amplitude of contraction?
 - What is the effect of the drug on the frequency of contractions?
 - What is the effect of the drug on tone of the uterine muscle?
- 2 For one drug:
 - Hypothesize a mechanism by which the drug affects the contractility of the uterine muscle.

Exercise 3: Length-Tension

Aim: To measure spontaneous contraction in the uterus stretched to different lengths; in this case, it is the same as being preloaded with different weights.

Procedure

- 1 Click **Start** and record spontaneous uterine muscle activity.
- 2 When the contraction cycles are consistent and predictable, use a ruler to measure the length of the uterus (from ligature to ligature) when the uterus is fully relaxed.
- 3 Type the relaxed length of the uterine muscle on the comment line and press the **Enter** key on the keyboard.
- 4 **Stop** to halt recording.
- 5 Select **Save** in the **File** menu,
- 6 Add more clay to the counterweight. More weight will increase the stretch or preload on the uterine muscle.
- 7 Repeat Steps 1 through 6, until the length of the relaxed uterus stops increasing or the amplitudes of the spontaneous contractions decrease.

Data Analysis

- 1 Scroll to the appropriate section of data for each relaxed length. Use the cursors to measure tone, contraction amplitude, period, and frequency.

- 2 Enter the data in the **Journal** and construct a table to display these parameters as a function of length.

Questions

- 1 Do the amplitudes of uterine muscle contractions depend upon muscle length?
- 2 Does uterine muscle tone depend upon muscle length?
- 3 Does the frequency of uterine muscle contractions depend upon muscle length?
- 4 Do your observations support the sliding filament theory for muscle contraction?
- 5 Do your observations supply evidence for plasticity?
- 6 How do your results compare to the length-tension relationship that exists in skeletal muscle?

Appendix

- 1 Tyrode's Physiological Saline, pH 7.4 with 6N HCl, in deionized water:

136.9 mM NaCl

2.68 mM KCl

1.80 mM CaCl₂·2H₂O

0.54 mM MgCl₂·6H₂O

100 mg% (mg/100ml) Glucose

10 mM Tris

- 2 Oxytocin in Tyrode's: 0.001g Oxytocin/100 ml. Use one drop; if no effect in 10 minutes, double the dose.
- 3 Acetylcholine in Tyrode's: 0.1g Acetylcholine Chloride/100ml. Use one drop; if no effect in 10 minutes, double the dose.
- 4 Atropine in Tyrode's: 0.1g Atropine sulfate/100ml. Use one drop, followed by a drop of the acetylcholine solution.
- 5 Epinephrine in Tyrode's; 0.01g L-Epinephrine/100ml). Use one drop; if no effect in 10 minutes, add another drop.

The following solutions are optional.

- 6 Leucine Enkephalin in Tyrode's: 0.01g Leucine Enkephalin/100ml. Use one drop.
- 7 Naloxone in Tyrode's: 0.01g Naloxone/100ml. Use one drop, after leucine enkephalin.
- 8 Methergine (Ergot Alkaloids) in Tyrode's: 0.2mg Methergine/ml. Use one drop.