# Small Animal EMG (Stimulation) General Setup

## Equipment

1. IX-RA5S Data Recorder.
2. iWire-BIO4 Recording Module.
3. A-UP5-DC-13-3A Power Supply Cable.
4. C-USB-C USB Cable Type C.
5. C-HVS-PN2 Platinum Needle Stimulating Electrodes.
6. C-ISO-PN5 Platinum Subdermal Needle Electrodes.
7. Heater Controller and Temperature Monitoring System

    option a) SA-TCS-6, A-TCT-1 (Mouse)

    option b) SA-TCS-8, TM-BIO (Rat)
8. MacOS or PC Computer system with LabScribe software installed.

## Supplies

1. Isoflurane inhalation anesthetics.
2. Masking tape.

## Overview

The Electromyogram (EMG) is recorded and analyzed with the iWorx data acquisition and analysis system. This system comes with the EMG Advanced Analysis Module, which allows for measurements of various EMG parameters such as Root Mean Square (RMS), Maximum Voluntary Contraction (MVC), Average Rectified Data, Threshold, Teager-Kaiser Energy, Mean and Median Frequency, Total Power, and more.

## Hardware Setup

1. Plug the IX-RA into a wall outlet using the provided power supply cord. The power port input is on the rear of the IX-RA.

2. Plug the IX-RA into the computer using the USB-C cable. The USB port is on the rear of the IX-RA.


*Figure 1: Rear view of the IX-RA5S.*

3. Plug the iWire-BIO4 recording module into the iWire Port 1 on the front of the IX-RA.

4. High Voltage Stimulation: Connect the stimulating electrodes (C-HVS-PN2) into the Red High Voltage Stimulator (HVS) ports on the IX-RA.


*Figure 2: Front view of the IX-RA5S. The RA5S has both low voltage and high voltage stimulators.*

5. Connect the recording electrodes (C-ISO-PN5) to the input channels of the iWire-BIO4.

***Note:*** *The colors of the electrode wires may not match the colors on the ports of the iWire-BIO4.*


*Figure 3: iWire-BIO4 Recorder with C-ISO-PN5 Recording Electrodes.*

6. Turn on the IX-RA using the power switch on the rear of the device.

## Software Setup

1. Download LabScribe software. Information on software installation can be found on the iWorx web page under Support → Getting Started: Software Installation - iWorx Systems Inc

***Note**: On the LabScribe Software Download Page, there is a tutorial video titled "LabScribe101 – Basic Training" This video is a basic introduction to LabScribe to learn how to set-up, record and analyze your data.*

2. Once installation is complete, open LabScribe software. A Device Status Dialog will pop up recognizing the devices.

3. Load the Research settings for the IX-RA.

Go To: Settings → Load Group → C:\Program Files\iWorx\LabScribe\Research Settings\RA5-Settings

Select: '**ResearchRA**' and click open.

4. Once the groups are loaded, select Settings → Click '**RS-EMG-SA-STIM**'

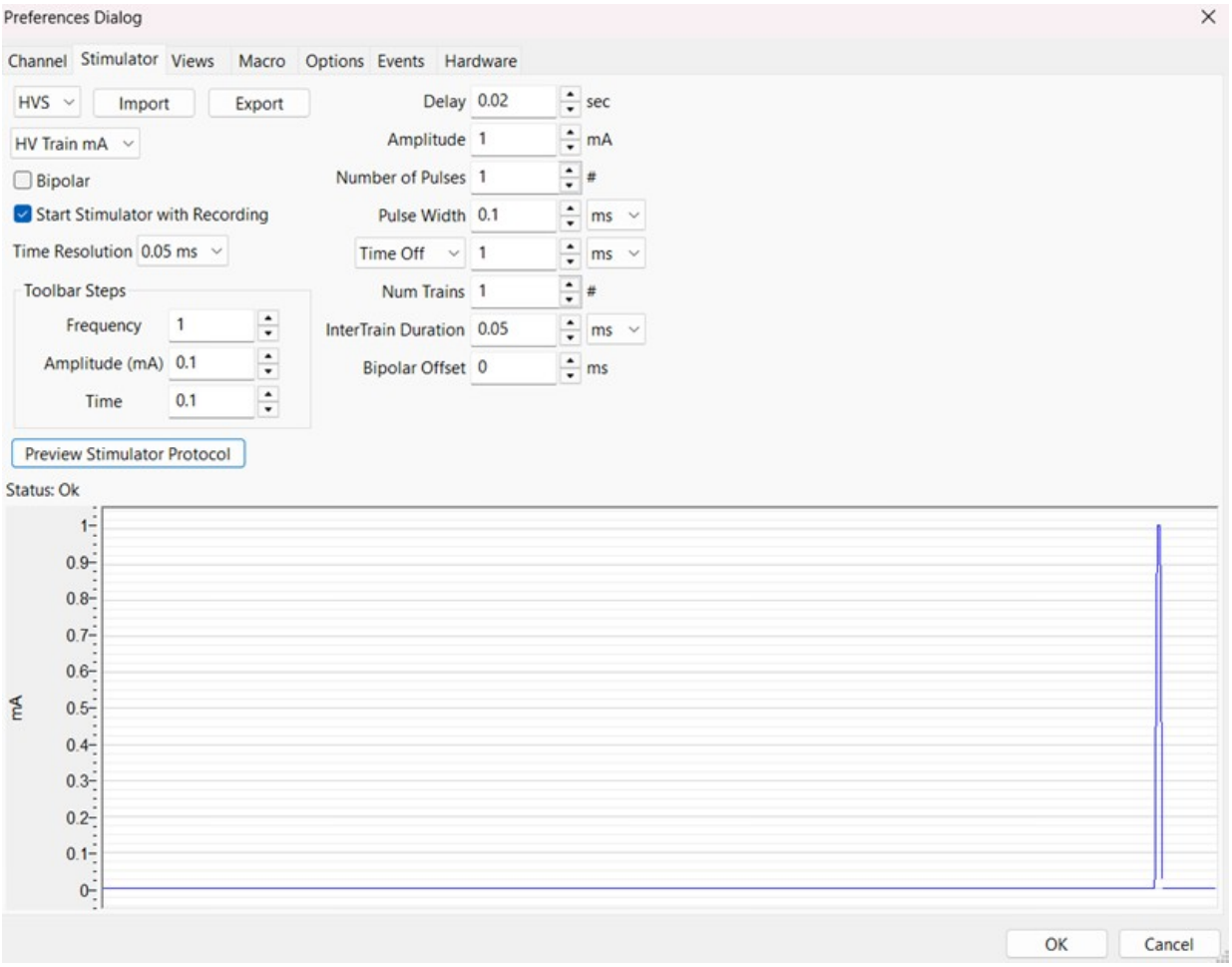
***Note**: Once the research settings are loaded, they will open the same every time LabScribe is launched. If you do not see the Research Settings folder in the LabScribe folder, reinstall LabScribe, making sure that the Research settings are installed.*

5. Configure the recording protocol.

Go To: Edit → Preferences → Stimulator

6. Set the stimulation parameters. They can be modified based on parameters for your individual experiment. (This settings file is set up for a series of stimulations with repetitively generated single square-wave pulses of 0.1 msec duration.)

- Current intensity: Start low (1 mA) and gradually increase, depending on experiment protocol.



## Animal Setup

Below describes an example protocol used to stimulate the sciatic nerve for a one-channel gastrocnemius muscle EMG recording.

1. Anesthetize the mouse following institution approved protocol (using 2–3% isoflurane for induction and 1–2% for maintenance).

2. Place the mouse on a heated surgical platform (SA-TCS-6) to maintain body temperature (37°C).

3. Clean the electrode placement sites using alcohol wipes.

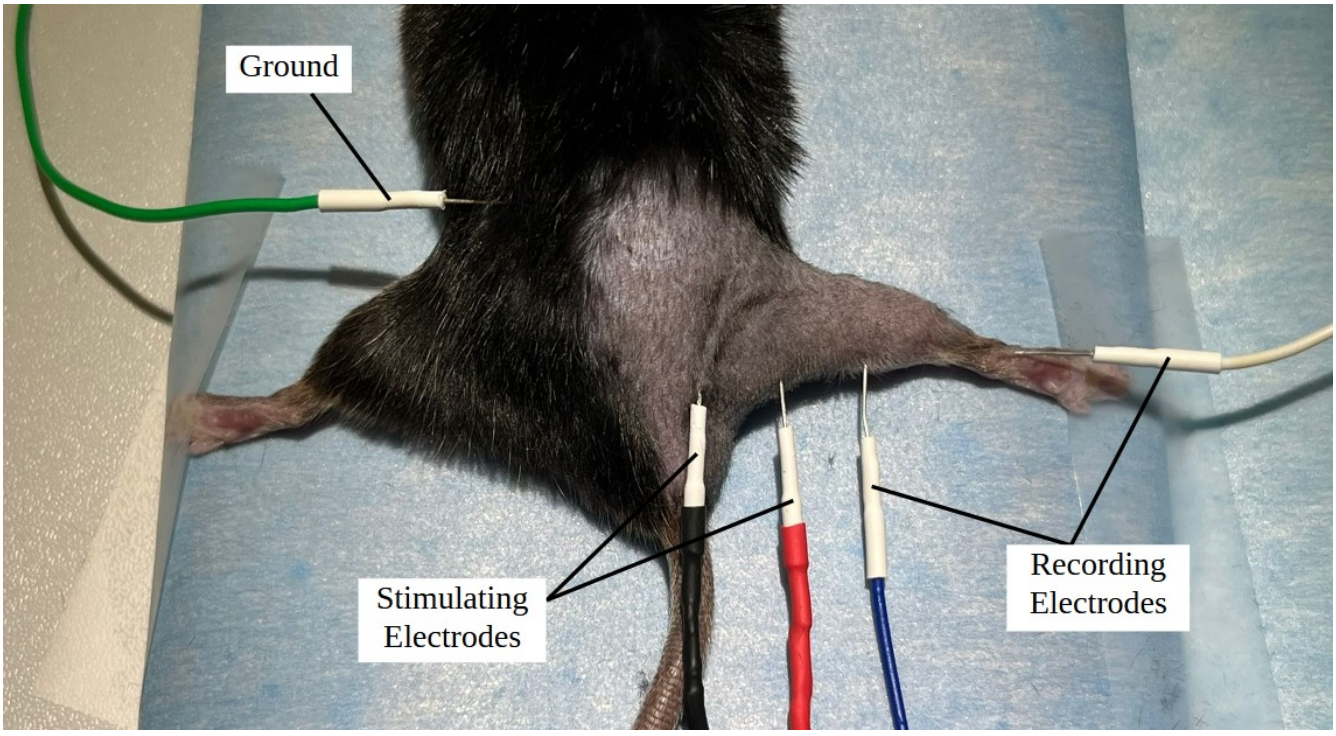
***Note:*** *Using needle electrodes for EMG recordings does not require shaving of the fur, but it may improve electrical conductivity.*

4. Insert the C-HVS-PN2 stimulating electrodes approximately 2 cm apart near the motor nerve of interest (sciatic nerve in the thigh).

5. Insert the C-ISO-PN5 recording electrodes for Channel 1 subdermally into the target muscle belly (gastrocnemius muscle).

6. Insert the green ground electrode (another C-ISO-PN5) into the base of the tail or above the muscle site to reduce electrical noise.

***Note****: EMG electrodes can be placed either subcutaneously or intramuscularly. One isn't necessarily preferred over the other as there are many factors that can contribute to which may be used. Although, subcutaneous placement is less invasive and is easier to apply with less risk of injury to the muscle tissues.*



# Recording

1. Initiate recording by pressing the red icon labeled "**REC**". The icon will now change to black and be labeled "**STOP**".


1. Select the 'AutoScale all Channels' button at the top of the toolbar. 

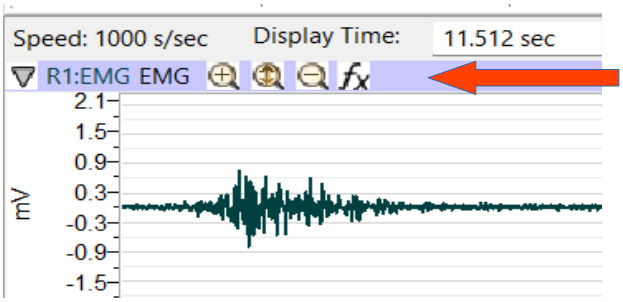
- This button can be pressed as many times as necessary throughout recording, or the recording can be continually AutoScaled in real time by selecting: View → Display → and Online AutoOffset. This will use the mean values of your data to adjust the trace view so you don't need to keep clicking Autoscale.

2. When the recording is finished Click the "**STOP**" icon.

***Note**: Confirm proper electrode placement by observing small muscle twitches with low-intensity stimulation. If necessary, slightly and carefully manipulate the position and / or the angle of the stimulation needle with respect to the nerve. Digital filtering techniques can also be applied to the signal in the LabScribe software post recording.*

# Labscribe Software Analysis


*Figure: Sample EMG Recording (Channel 1 – Dark Green) and Muscle Force (Channel 2 – Light Green). Computed channels off of the EMG channel are shown in Blue, consecutively: MVC, RMS, Threshold.*



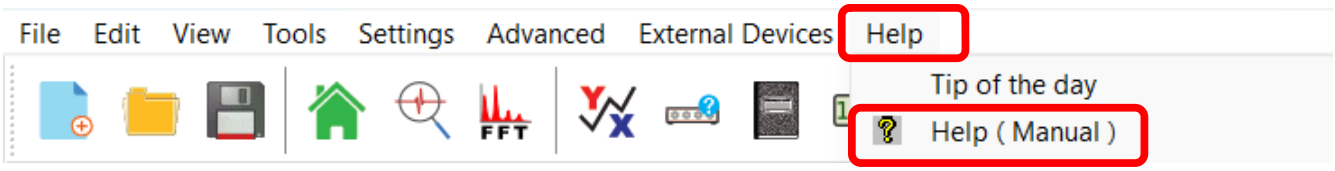

The fx icon allows for the creation of a new channel with the selected function.

1. Select ‘fx’
2. Select ‘EMG’
3. Select the type of analysis you would like to perform on the selected channel.

To open the available analysis options in the Advanced EMG Analysis Module, select: ‘fx’ → EMG.

Detailed information on EMG Analysis and the Advanced EMG Analysis Module can be found in the LabScribe manual by navigating to:

- In the LabScribe toolbar Select: Help → Help (Manual)
- EMG Analysis Section 8.18



## Reducing Noise

- Make sure that the heater used for maintaining the animal is a shielded low noise heater.
    - The iWorx SA-TCS-6 or SA-TCS-8 system is designed to minimize noise from the heater.
- To minimize noise from the heater bed, wrap the electrode wires in tinfoil, reduce their length, and braid them to limit power supply noise pickup.
- Utilize a grounding cable to connect the recorder to the wall outlet for better noise reduction.
- Make sure the recording computer is plugged into the wall outlet, not just running on battery power.
- Turn off any excess overhead lights, lamps, and electronics in the lab. Vibrating equipment like pumps, fans, or even nearby footsteps can also affect recordings.
- The ground electrode may need to be adjusted. Try moving the electrode above the muscle site, below, or at the base of the tail.