Manual for Rodent Nerve Stimulators

Harald M. Stauss, MD

Version 3

July 18, 2017

Contents

Introduction

The nerve stimulators consist of three important parts: (1) the battery, (2) a microprocessor-operated stimulator module, and (3) stimulation electrodes. To some degree, all three parts can be customized according to the specific needs of the experiments.

Stimulation Mode

The stimulators work as “constant voltage” stimulators. The stimulator module generates rectangular impulses of a specific frequency, pulse width, and voltage. These rectangular impulses are delivered to the positive electrode (anode) through a capacitor to achieve charge-balanced stimulation. The negative electrode (cathode) is tied to ground potential. Thus, relative to the anode, the cathode generates a charge-balanced negative impulse (see Fig. 1). This negative impulse under the cathode causes depolarization of the nerve fibers and initiates an action potential. At the anode, the positive impulse will hyperpolarize the nerve fibers and, theoretically, would block nerve conduction. However, practical experiments with stimulation parameters within the range of the stimulators demonstrated that the orientation of the anode and cathode along the nerve (i.e., distal or proximal position of anode vs. cathode) does not make a difference in the excitation of the nerve and anodal blocking did not occur (1). Thus, for practical purposes, the placement of the anode vs. cathode along the nerve is not relevant and the electrodes of the stimulators are not marked as anode or cathode.

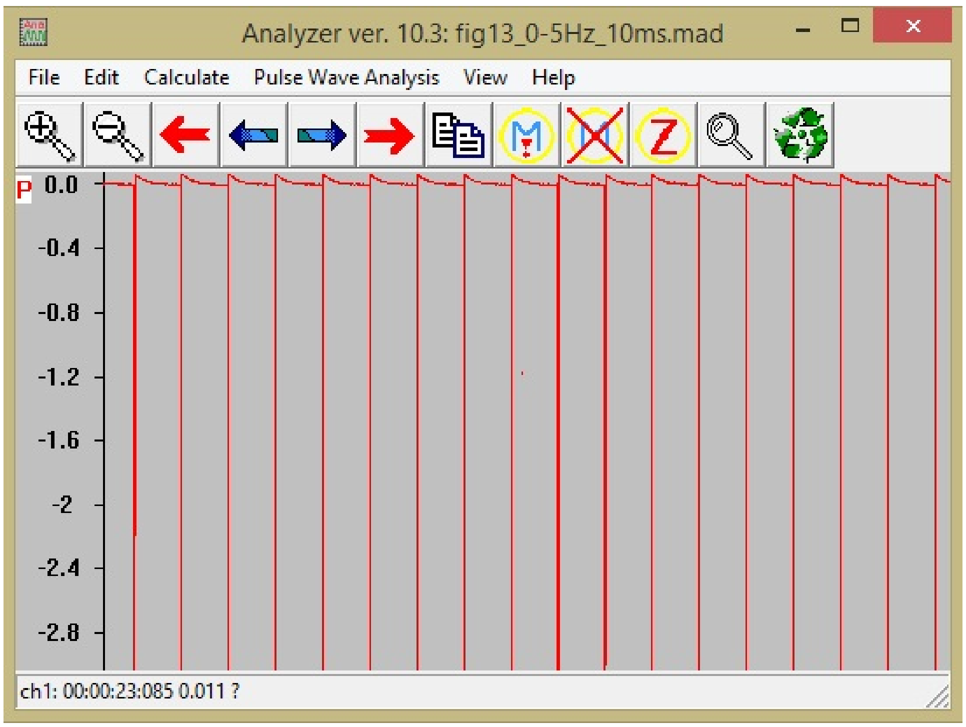

Fig. 1: Stimulation at 5Hz, 3V, 1ms pulse width. Note the brief periods of positive voltage following each impulse that effectively charge-balance the stimulation. In this example the voltage at the cathode was recorded relative to the anode. Thus, the impulses show up as negative voltages that depolarize nerve fibers under the cathode.

Stimulation Parameters

At the time of manufacturing, the stimulator modules are programmed with six different sets of stimulation parameters depending on the needs of the experiments. The stimulation voltage is determined by the battery used and, therefore, is always the same for all six sets of stimulation parameters. However, the stimulation frequency, pulse width, and duty cycles can be programmed differently for each of the six sets of stimulation parameters.

Stimulation Voltage

The stimulation voltage is defined by the battery used in the stimulators. For rats the options are 3V, 3.6V, or 6V. For mice, the small size of the stimulators only allows for the use of 3V batteries. Thus, the mouse stimulators can only deliver impulses of 3V.

Stimulation Frequency

For stimulating nerve fibers commonly used stimulation frequencies range from 1 Hz to 30 Hz. For blocking nerve traffic stimulation frequencies in the kilohertz range (e.g., 5,000 Hz) have been used (2). The stimulators can be programmed with stimulation frequencies ranging from 1 Hz to the kilohertz range.

Pulse Width

By varying the pulse width it may be possible to recruit different fiber types. For example, our previous study (1) suggested that at relatively low stimulation frequencies (<5 Hz) and short pulse widths (<500 µs) primarily larger fibers are recruited, whereas at longer pulse widths (>500 µs) smaller B and C fibers may also be recruited.

Duty Cycle

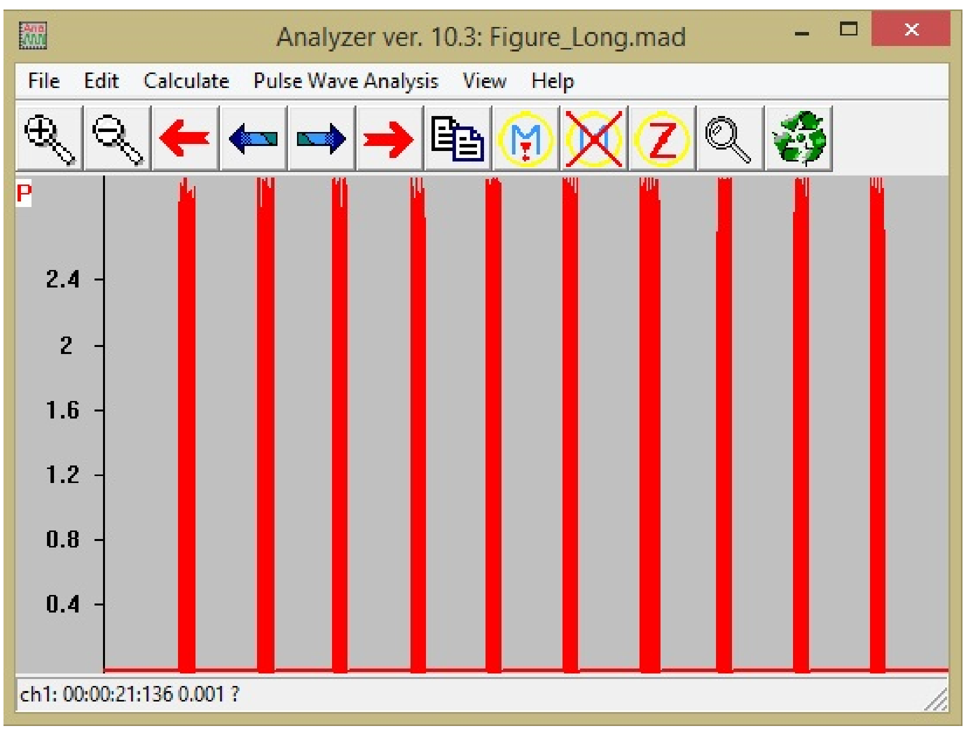

Fig. 2: In this example the duty cycle was set to alternating cycles of 5 s on and 25 s off.

The duty cycle defines alternating cycles where the stimulator is automatically turned on and off. For example in Fig. 2, the stimulator was programmed to turn on for 5 s and then off for 25 s. For each of the six sets of stimulation parameters different (or the same) duty cycles can be programmed. In a previous study (3) we programmed the duty cycles for 1 h on and

1 h off. In this study we also recorded arterial blood pressure and heart rate along with vagal nerve stimulation. Due to the duty cycle, blood pressure and heart rate oscillated in synchrony with the duty cycle (e.g., heart rate dropped for one hour when the stimulators turned on). These oscillations in blood pressure and heart rate could then be used to verify the biological response to the stimulation.

Electrodes

After many experiments, we found that multi-stranded stainless steel electrode wire works best. Single-stranded wire has a tendency to break and generally is stiffer than multi-stranded wire. For rat stimulators we generally use Catalog #: 793500 and for mouse stimulators Catalog #: 793400 from A-M Systems (https://www.a-msystems.com/p-806-multi-stranded-pfa-coated-stainless-steel-wire.aspx). These are PFA coated multi-stranded stainless steel wires. We also found that using coil electrodes (see Fig. 3) that are wrapped around the nerve work best. For that, we remove the PFA insulation at the tip of the wire and wrap the un-insulated tip of the wire around injection needles to shape the coils. We use different sizes of injection needles for different nerves. For example for the vagus

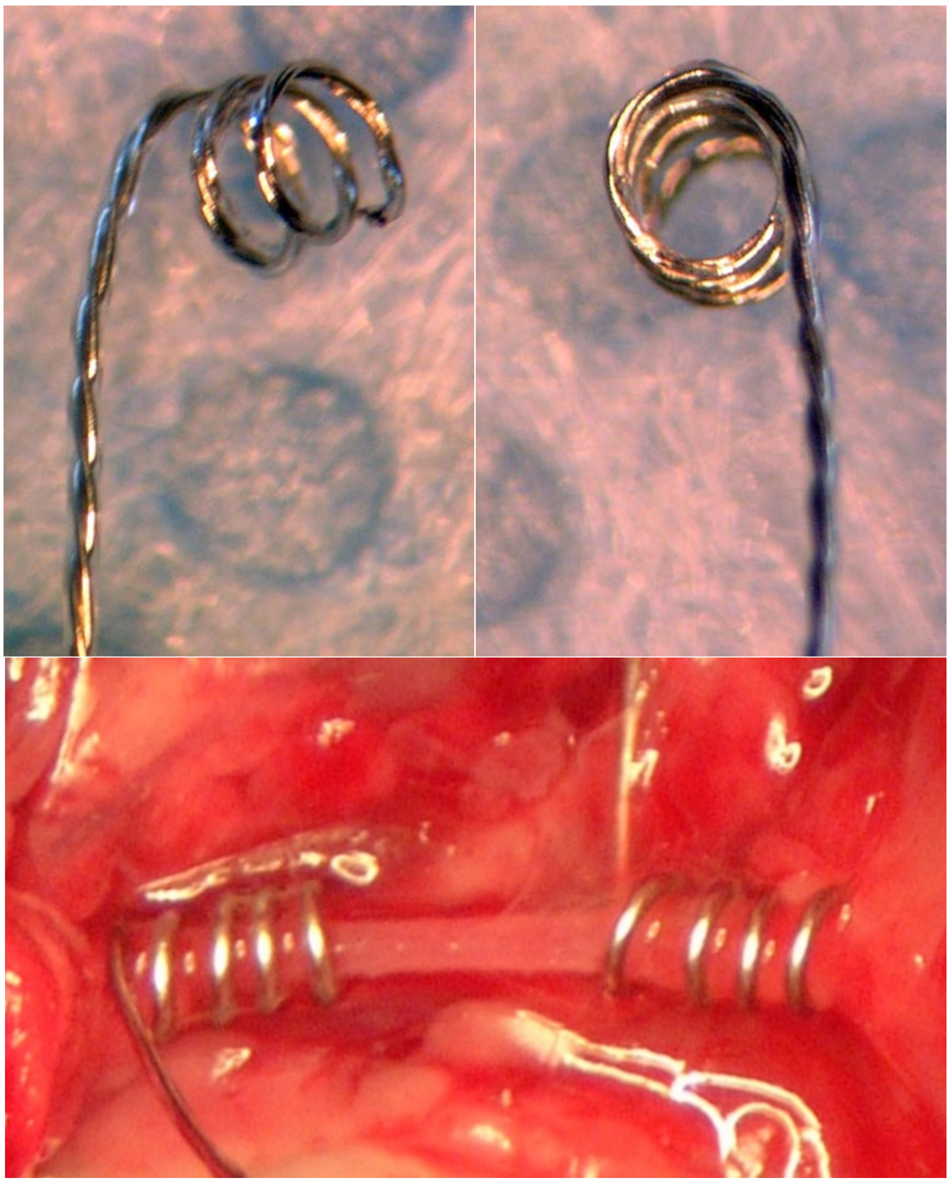

Fig. 3 top: Coil electrodes for mouse implants. These coils are made of A-M Systems Catalog #: 793400 electrode wire. This is a PFA-coated, 3-stranded stainless steel wire. The inner coil diameter is 23G (=0.64 mm).
Fig. 3 bottom: Coil electrode wrapped around the common carotid artery in a mouse. The vagus nerve is located below the artery (not visible) but inside the coil electrodes. Here a single-stranded stainless steel coil electrode was used.

nerve in rats, we generally use a 25G needle. For the vagus nerve in mice, we use a larger 23G needle, because in mice we leave the vagus nerve attached to the common carotid artery and wrap the coil electrode around the artery and nerve. This way, the artery stabilizes the nerve preparation. For other nerves, different sizes of coils can be used.

Turning Stimulators On and Off and Selecting Stimulation Settings

The stimulators are delivered in the off position. When turned off, the battery drainage is minimal and the stimulators are in a “stand-by” mode and can be activated using an externally applied magnet. A larger size magnetic stirring bar for laboratory use works well. When the magnet is swiped one time along the battery housing of the stimulator, the stimulator turns on and will be operating at the first set of stimulation parameters. The stimulators also house a radio transmitter that will transmit a signal that indicates the setting of the stimulator. The radio signal can be received with a small transistor radio tuned at a frequency of 660 kHz in AM mode. When the stimulators are turned on, one beep (short audio signal) will be heard in the radio, indicating setting 1. With subsequent swipes of the magnet, the stimulators will go into setting 2, 3, 4, 5, and 6 which will be indicated by 2, 3, 4, 5, or 6 beeps heard in the radio, respectively. These beeps will be repeated at the beginning of each duty cycle or every 60 seconds, whichever, is first. Thus, if the stimulators are not turned off, an audio signal (beeps) can be heard at least every 60 seconds. Once setting 6 is reached, another swipe with the magnet will activate a mode where the stimulator transmits a constant audio signal. This mode is included to allow to fine tune the transistor radio to the exact frequency of the radio transmitter inside the stimulator. *The stimulator should not be in the constant radio signal mode for too long, because the constant radio transmission can rapidly drain the battery.* Another swipe with the magnet will turn the stimulator off (low battery drainage, stand-by setting, no audio signals received in radio). It is advised to check the setting of the stimulators on a daily bases during the duration of an experiment.

Important: We had one instance, where a stimulator was turned on during shipping because it was exposed to a magnetic field. Thus, upon receipt of the stimulators, immediately make sure that the stimulators are turned off. Turning off the stimulators, will preserve the battery life. It is best to cycle the stimulators through the six settings, constant radio signal, and then turn them off until they are used. If a stimulator does not turn on upon receipt (i.e., the stimulator is defective), please send it back and it will be repaired or replaced free of charge.

Sterilization

Before shipping, the stimulators are sterilized using 12 hours of Ethylene Oxide gas sterilization at 130°F. Sterilization should also be possible by immersing the stimulators in 2% Glutaraldehyde (Cidex®) solution for a minimum of 10 hours. Before implantation, the glutaraldehyde must be washed off the stimulators using sterile saline solution. However, there is a risk that glutaraldehyde may penetrate into the stimulator battery housing causing severe damage to the stimulators. Gas sterilization is preferred.

Refurbishing Stimulators

Once an experiment is completed the stimulators should be explanted taking care not to damage the electrode wires. If the stimulators are still working (as indicated by radio signal transmissions), the coils of the electrodes can be reshaped and the stimulators sterilized for use in another animal. This applies particularly to the rat stimulators (and not as much to the mouse stimulators) because the rat stimulators have larger batteries and, thus, longer battery lifes than the mouse stimulators. If the battery is dead (no radio signal transmission) or the electrode wires are damaged, the stimulators should be shipped back for refurbishing (for a nominal fee). Refurbishing includes opening the stimulators, cleaning the circuit board, attaching a new battery and new electrode wires, and sealing the stimulators. Some investigators (including us) have used stimulators with dead batteries as "dummy stimulators" for "sham stimulation" in control groups of animals.

References

1. **Stauss HM.** Differential hemodynamic and respiratory responses to right and left cervical vagal nerve stimulation in rats. *Physiological reports* 5, 2017.
2. **Patel YA and Butera RJ.** Differential fiber-specific block of nerve conduction in mammalian peripheral nerves using kilohertz electrical stimulation. *Journal of neurophysiology* 113: 3923-3929, 2015.
3. **Chapleau MW, Rotella DL, Reho JJ, Rahmouni K, and Stauss HM.** Chronic vagal nerve stimulation prevents high-salt diet-induced endothelial dysfunction and aortic stiffening in stroke-prone spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 311: H276-285, 2016.