StimJim Isolator Manual

(Version 1.00)

by Willaim W. Anderson, WinLTP Ltd.

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The copyright of this manual and the StimJim_Isolator program code is the same as Nathan Cermak's StimJim software, ie CERN Open Hardware Licence v1.2, and is therefore free and Open Souce.

Additonal code in StimJim Isolatory by Nathan Cermak and Philippe Lucidarme is free and Open Source. The WinLTP code included in the StimJim_Isolator program (copyrighted by WinLTP Ltd. and The University of Bristol) is also free and Open Source

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CHAPTER 1 – Background

1.1 Introduction

StimJim_Isolator is a Windows program that controls the StimJim stimulator to act like an intelligent stimulus isolator. One input pulse from your data acquisition program triggers one isolated mono, bi, or triphasic current or voltage pulse from the StimJim. The 'intelligence' is because pulse amplitudes can be automatically incremented by just clicking one button (Fig. 1.1.1).

The StimJim_Isolator program is free and, like the StimJim, is open source.

The 2 channel <u>StimJim</u> was designed by Nate Cermak and Jon Neuman of the Open Ephys group, manufactured by <u>LabMaker</u>, and sold for \$645 USD. The documentation for the StimJim is in the bioRxiv paper (Cermak N, Wilson MA, Schiller J and Newman JP (2019) Stimjim: open source hardware for precise electrical stimulation. bioRxiv, doi: <u>https://doi.org/10.1101/757716</u>).



Fig. 1.1.1. Clicking the **'Enable Incrementing'** button causes 5 incrementing pulses to run. After incrementing finishes the constant amplitude pulses resume. Incrementing can be stopped at any time by clicking the **'Enable Triggering Constant Pulses'** button, or the **'Stop'** button.

1.2 Major Limitation of StimJim - Low Compliance Voltage, and Electrodes that Will Work with StimJim

I have found only one major limitation with the StimJim – the low compliance voltage of about \pm 13.7 volts. So a 100 Kohm stimulating electrode can only output a pulse with a maximum of 137 uA. This means that, in general, the StimJim cannot use the higher resistance metal electrodes like the Haer 30203, but only low and medium resistance electrodes such as the:

a) Haer 30202 and 30213 (Bipolar Concentric, 25um Cone Tip, Platinum/Iridium) Electrodes

b) 50um Platinum/Iridium Bipolar Twisted Wire Electrodes

(see <u>Sections 2.6</u> and <u>4.1.</u>

It's important to realize that the StimJim / StimJim_Isolator is one of the few stimulus isolators that can measure the maximum passible current and electrode resistance during the stimulation pulse when the electrode is in the tissue as well as in the saline.

Using these measurements, we found that:

- a) with substantial stimulation, the resistance of new electrodes could about double even with biphasic pulses.
- b) when the stimulating electrode in the saline was pushed into the slice the the resistance could sometimes double or even quadruple (like going from 16 to 69 Kohms). I thought this result was suprising, because I had thought the extracellular resistance was only a couple of Kohms.

If you find that your stimulating electrode is not passing enough current, you can 1) try a longer stimulus duration, such as increasing from 100 to 150 or 200 us, or 2) try a lower resistance electrode.

1.3 Description of the StimJim

The StimJim used in the development of StimJIm_Isolator.exe is version 0.18 and purchased June, 2021.

First of all, see this research paper for StimJim details (Stimjim: open source hardware for precise electrical stimulation (2019) Nathan Cermak, Matthew A. Wilson, Jackie Schiller and Jonathan P. Newman, *bioRxiv*, doi: https://doi.org/10.1101/757716).

From LabMaker, the StimJim comes with its own USB Micro-B to USB cable, so hardware-wise, its ready to go.

The StimJim has two output channels and two input channels. It is very inexpensive - \$645 USD for 2 channels or \$325/channel (Figs. 1.3.1 and 1.3.2).

The two output channels are largely, although not entirely, independent (see Appendix 1.2).

The StimJim can output isolated constant current or constant voltage. To my knowledge, most extracellular stimulation uses constant current because the amount of current delivered will theoretically not change if the resistance of the stimulating electrode changes for whatever reason including electrode polarization.

The StimJIm can output monophasic, biphasic and triphasic current pulses, ie with or without an inter-gap period (see Fig. 3.1.3.1). Furthermore, the last phase of the stimulation can be of reduced amplitude and longer duration so as to make sure the first phase of the stimulation is the phase stimulating the axons.

Importantly, the StimJim grounds out the output between pulses. This stops leakage current between pulses which can be a problem with stimulus isolators which do not have this capability. This biphasic current pulse output plus grounding out the output between pulses goes a long way to eliminating electrode polarization and insuring reliable constant stimulation.

As discussed above, the compliance voltage is on the small side, ± 13.7 volts per channel. This may seriously limit the amount of current your stimulating electrode can pass. Most other stimulus isolators have compliance voltage of between ± 44 to ± 100 volts.



Fig. 1.3.1. The front panel of the StimJim showing Channel 0 and Channel 1 outputs (both a BNC connector and 2 + and – pins), and Input 1 and Input 2 BNC connectors.



USB Connector

GPIO Connector

Fig. 1.3.2. The back panel of the StimJim showing the delicate USB Micro-B connector, the two green LED lights that illuminate when the StimJim is initially plugged into the computer and when pulses/trains are output, and the General Purpose I/O (GPIO) 16 pin connector.

1.4 Advantages of Using the StimJim

There are several advantages to the StimJim programmable stimulus isolator over the Digitimer DS4 and other Voltage-controlled stimulus isolators and programmable stimulus isolators.

- 1) Cost. A two channel StimJim costs \$645 USD (\$325/ch), whereas other voltage-controlled stimulus isolators cost several times that amount (\$1700 to \$2100 / channel). For multislice setups with 4-8 stimulating electrodes, this cost difference can be significant.
- 2) For many non-gated Voltage-controlled stimulus isolators, the number of isolators you can use is limited by the number of analog outputs on the data acquisition board (usually 2 or 4). For some digitally gated voltage-controlled stimulus isolators, like the Digitimer DS4, whether an output occurs or not can be controlled by digitally gated input. So one analog output can be used control many digitally gated voltage-controlled stimulus isolators.

- Also, the StimJim grounds the current output between pulses (<u>Appendix 3</u>). Not all voltage-controlled stimulus isolators do this, and therefore with those isolators there could be current leakage between pulses.
- 4) The StimJim is powered by a USB cable, so concerns about battery rundown during an experiment do not exist.

1.5 Reasons to Upgrade from Beta Version 0.12

If you have been using the StimJim_Isolator (beta version 0.12) there are several reasons to upgrade to 1.00:

- Version 1.00 measures the current passed during *each* pulse and if it is substantially lower than the requested current (by 10%), this indicates that the compliance voltage (of approx. <u>+</u>13.7V) and the maximum current passable has been reached, and is output as an error.
- 2) Version 1.00 increments of Chs 0 and 1 can be run simultaneously, although 100 ms should be between Ch0 and Ch1 pulses.
- 3) The screen space taken up by the StimJim Isolator version 1.00 is more compact, all panels can be put inline to view all at once rather than in separate tabsheets, and two channels can be viewed above/below each other as well as side-by-side (<u>Section 2.7</u>).

1.6 StimJim Isolator is Completely Independent of WinLTP

Although the StimJim Isolator program was written by the developer of WinLTP (Bill Anderson), and therefore works perfectly well with WinLTP, the StimJim Isolator program also is *completely independent* of WinLTP and *should work with almost all electrophysiology data acquisition systems*.

The only requirement of the data acquisition system is that a digital pulse from the data acquisition system has to trigger each single pulse. Programs capable of doing this include Molecular Devices' pClamp and John Dempster's WinWCP.

1.7 Changing the StimJim Isolator Using C++Builder

Because the StimJim design is free and open source, I decided that the StimJim Isolator program should also be free and open source. The source code for version 1.00 and more developer information (including a compiled version of stimPulserClient.exe) is included in the download.

The StimJim Isolator program, like WinLTP, uses the Embarcadero/Borland C++Builder compiler. The Embarcadero/Borland C++Builder is not as popular as Microsoft's Visual C++, but it is available as a <u>free</u> <u>'community' version</u> for developers creating Open Source software. The StimJim_Isolator program also uses a component library LMD-Tools from LMD Innovative that is not free but costs around 200 Euros. The StimJim_Isolator program uses some code from WinLTP, and this code is now open source and free. This WinLTP code in particular uses LMD C-Tools to control edit fields, so it is very important in the StimJim_isolator.

CHAPTER 2 – Installation and Startup

2.1 Installing One Program

To install one program, download the installation program, Install_StimJimIso100.exe, from the webpage <u>winltp.com/StimJim_Isolator.html</u>, and double-click on it to run it.

The installation program will create a folder:

C:\Program Files (x86)\StimJim_Isolator\

or (if administrative rights are difficult to get), you can change it to:

C:\StimJim_Isolator\

The installation program will next copy the files:

StimJim_Isolator100.exe program

StimJim_Isolator100_Manual.pdf file

StimJim_Isolator100_Developers.zip

into the \StimJim_Isolator folder, and make shortcuts to place StimJim_Isolator100.exe and StimJim_Isolator_Manual.pdf on the Desktop.

The StimJim_Isolator100_Developers.zip file contains the:

stimPulserClientBA.exe program (a ready to use version of Nate Cermak's stimPulserClient.exe) StimJim_Isolator100_DevelopersCode folder containing:

StimJim_Isolator100_Manual folder with docx and png files StimJim_Isolator100k_Code folder with all the Embarcadero C++Builder code StimJim_Isolator012_Package.zip file containing beta version exe code and **test data**

When the StimJim_Isolator100.exe program starts, a folder:

C:\Users\Public\Documents\StimJimIso\StimJimIso1

is automatically be created, andwhere protocol and log files will be saved.

2.2 Installing Two to Eight Programs

Up to 8 StimJims and StimJim Isolator programs can be run on one computer. To install the second StimJim / StimJim Isolator and more, you have to copy and the StimJim_Isolator100.exe program and rename it StimJim_Isolator2.exe etc into the same \StimJim_Isolator folder. Renaming the program files will cause them to save the protocol and log files to a different folder:

Program Name

- StimJim_Isolator100.exe StimJim_Isolator2.exe StimJim_Isolator3.exe
- StimJim Isolator4.exe
- _____StimJim_Isolator5.exe
- · · · · · · · · · · · ·
- StimJim_Isolator6.exe
- StimJim_Isolator7.exe
- StimJim_Isolator8.exe

- will save protocol and log files to this folder:
 - C:\Users\Public\Documents\StimJimIso\StimJimIso1
 - C:\Users\Public\Documents\StimJimIso\StimJimIso2
 - C:\Users\Public\Documents\StimJimIso\StimJimIso3
 - C:\Users\Public\Documents\StimJimIso\StimJimIso4
 - C:\Users\Public\Documents\StimJimIso\StimJimIso5
 - - C:\Users\Public\Documents\StimJimIso\StimJimIso6
 - C:\Users\Public\Documents\StimJimIso\StimJimIso7
 - C:\Users\Public\Documents\StimJimIso\StimJimIso8

Then again make a shortcut to StimJim_Isolator2.exe, place on the Desktop, and rename to "StimJIm Isolator 2". Repeat this process for 3 up to 8 programs. Note that each program has a different background color and StimJim_Isolators 2 to 8 have the program name "SJI" plus the program number, ie "SJI2" on the title bar (Fig. 2.2.1).

Also, when you are using multiple StimJims, it is useful to use a USB hub where the individual StimJims can be easily connected and disconnected (Fig. 2.2.2).



Fig. 2.2.1. Eight StimJIm Isolator programs running simultaneously, each with a different background color, and the program name and number, ie "SJI2" in the title bar. One one channel per program is shown there.



Fig. 2.2.2. Using a USB hub to connect to multiple StimJims.

2.3 Starting All Other COM Programs First

The StimJim_Isolator program connects the StimJim to the first available COM port. Therefore, if there are other program that you use in your computer (like Scientifica's LabLink) that use already defined ports, start those program first so that the already defined COM ports are already used.

2.4 Starting the First StimJim – Connecting to a COM Port

When the StimJim is connected to the computer by the USB cable, Windows creates a serial COM port.

To unambiguously determine this COM port number:

- 1) Open the Device Manager (right mouse button click the Windows '**Start**' button and then click on the **'Device Manager'** menu item.)
- 2) If necessary, unplug the USB cable to the StimJim, then
- 3) Replug the StimJim USB cable into your computer. These lines will appear in the Device Manager:



The number after 'COM' (in this case 3) is the serial port NUMBER that THIS StimJim is connected to. COM1 and COM2 are usually legacy hardware COM ports – and NOT used as the the StimJim port.

When StimJim_Isolator.exe is started, the program shows one of two situations. If a serial COM port is detected, then the main form of the program is shown with the "Successfully connected to ComX" in white/green in the Ch0 status bar (Fig. 2.4.1, red arrow).

If a serial COM port is NOT detected – probably because the StimJim is not plugged in to your computer's USB port) - then the 'Set Serial Port' tabsheet of the 'Setup' dialog is shown (Fig. 2.4.2). If the 'Set Serial COM Port' combo box is empty, again, this probably means that the StimJim is NOT CONNECTED to your computer.

To connect to correct COM port:

- 1) Click the **'Retry Detecting Serial Ports'** button to see if this program can now detect the StimJim serial port
- 2) In the 'Set Serial COM Port' combo box select the COM# seen when the StimJim was plugged in.
- 3) Then click the 'Try Connecting StimJim to Serial Port' ', and this message should appear:

If you find that the initial COM port number is incorrect, you can also call up the '**Setup**' dialog by clicking on the 'hamburger' icon in the upper left and clicking on the '**Run Setup Dialog...**' menu item (Fig. 2.4.3).



Fig. 2.4.1. The way StimJim_Isolator program appears if there is a successful connection to the COM serial port which elicits the Ch0 status bar message in white/green "Successfully connected to COMX" (red arrow). The default protocol name is displayed in the title bar ("SjDefault.pro"), and temporarily in the Ch1 status bar. The default Current/Voltage mode is "Current (uA)" so that you can test the maximum current the stimulating electrode can pass, and its approximate resistance. The 'Evoke Single Train' panel is not shown.

Setup	×
Set Serial Port Number of Channels Layout and Log/Increment	
If the Serial COM Port is blank, plug in the StimJim Set the Serial COM Port COM3 v 1) Retry Detecting Serial Ports 2) Try Connecting StimJim to Serial Port	Set the StimJim Serial COM Port When the StimJim is connected to the computer by the USB cable, Windows creates a serial COM port. If the 'Set Serial COM Port' combo box is empty, this probably means that the StimJim is NOT CONNECTED to your computer. To unambiguously find, and connect to, the COM port: 1) Open the Device Manager (right mouse button click the Windows start button and then click on 'Device Manager' menu item.) 2) If necessary, unplug the USB cable to the StimJim, then 3) Replug the StimJim USB cable into your computer. These lines will appear in the Device Manager: Ports (COM and LPT) USB Serial Device (COM3) The number after 'COM' is the serial port NUMBER that THIS StimJim is connected to. (COM1 and COM2 are usually hardware COM ports - not the StimJim port.
StimJim Bootup Information Booting StimJim on Teensy 3.5! Initializing inputs AOC offsets (+-2.5V): -9.000000, -10.960000 ADC offsets (+-10V): -8.350000, -10.140000 current offsets: 3, -8 voltage offsets: 0, 0 Ready to go!	 4) Click the 'Retry Detecting Serial Ports' button to see if this program can now detect the StimJim serial port 5) In the 'Set Serial COM Port combo box select the COM# seen when the StimJim was plugged in. 6) Then click the 'Try Connecting StimJim to Serial Port' button, and this message should appear: "Successful connection to COM#"

Fig. 2.4.2. 'Set Serial Port' tabsheet in the 'Setup' dialog.

👎 SJI SjDefault.pro		- 0	×
Channel 0	Ξ	Channel 1	
Evoke Single Pulse		Open Protocol File Save Protocol File	e
Enable Triggering of Constant Amp Pulses		Save Protocol File As Set To Default Protocol	of
Current or Voltage Current (uA)		Run Setup Dialog	uA) 🗸
20 Pulse Amplitude (uA) 20 Manual Increment (uA) 20 Manual Increment (uA)		Always On Top About Exit	(uA)
100 Pulse (Phase0) Duration (us 0 Inter-Phase1 Duration (us) 2 Phase2 Dur (x0-x4) 200us)	100 Pulse (Phase0) Duration 0 Inter-Phase1 Duration 2 Phase2 Dur (x0-x4) 2	on (us) (us) 00us

Fig. 2.4.3. 'Run Setup Dialog...' menu item.

2.5 Starting More Than One StimJim

I have not been able to get a unique serial number from the StimJim. Because of this you have to be careful in unambiguously associating a particular StimJim Isolator program running on ONE computer (and the protocol file running on that program) with a particular StimJim (and the particular stimulating electrodes connected to it).

So a kludge that should work is

- 1) Before you start any StimJim Isolator programs, disconnect all StimJim USB cables from your computer (it would be easiest to use a USB hub with On/Off buttons for this purpose).
- 2) Connect the StimJim1 USB cable to the one computer to turn StimJim1 on, then start the StimJim_Isolator100.exe program to connect it to the first COM# available (COM3?).
- 3) Then connect the StimJim2.exe USB cable to the one computer to turn StimJim2 on, then start the StimJim_Isolator2.exe program to connect it to the first COM# available (COM4?).
- 4) Repeat this process for the remaining StimJims.

2.6 Setting the Number of Channels (and Compliance Voltage Information)

The 'Number of Channels' tabsheet in the 'Setup' dialog (Fig. 2.6.1) is set by the button group in the upper left of the tabsheet. You can use Ch0 only, Chs 0 and 1 independently, or Chs 0 and 1 in series to produce one output. For the first two choices, the compliance v

oltage is ± 13.7 volts in Current Mode and ± 15 volts in Voltage Mode. When using Chs 0 and 1 in series, in Voltage Mode the output will double to ± 30 volts, but in Current Mode, the compliance voltage remains at ± 13.7 volts, not the ± 27 volts as stated in the Cermak et al. 2019 bioRxiv paper (see <u>Appendix 1.1</u>).

This ± 13.7 volts compliance voltage is lower than most stimulus isolators, and it means that the StimJim can only be used with medium and low resistance stimulating electrodes, not the high resistance electrodes (see <u>Sections 1.2</u> and <u>4.1</u>)

You should know the maximum amount of current you need to stimulate with. Most slice stimulation requires a maximum of 50 to 500 uA. But you probably don't know the resistance of your stimulating electrode to a 100 usec or 1 msec stimulation.

The maximum amount of current an electrode of a given resistance can pass is determined by the compliance voltage:

Imax = Vcompliance / Relectrode

To determine the maximum amount of current stimulation electrode can pass and its approximate resistance, try passing a large amount of current that will cause the compliance voltage to be reached (<u>Section 3.5</u>).

Setup	×
Set Serial Port Number of Channels Layout and Log/Increment	
Number of Channels and Compliance Voltage	Number of Chs and Compliance Voltage
Choose Layout, Number of Channels and Compliance Voltage Using Ch 0 only Using Ch 0 only Compliance Voltage 13.7V, In Voltage Mode 15V Using as one output, Chs 0 and 1 in series Compliance Voltage 13.7V, In Voltage Mode 30V	You should know the maximum amount of current you need to stimulate with. Most slice stimulation requires a maximum of 30 to 500 uA. The maximum amount of current an electrode of a given resistace can pass is determined by the compliance voltage (the maximum voltage an isolator can pass in current mode): Imax = Vcompliance / Relectrode
Compliance Voltage of Some Stimulus Isolators NPI Iso-Stim-II +/.100V (analog & digital controlled, biphasic) Upi A365 +/.100V (digitally triggered, biphasic) Digitimer DS3 90V (digitally triggered, biphasic) MultiChannel Systems STG-400x +/.60V (program controlled, biphasic) A-M Systems 2200 and 2300 +/.50V (analog & digitially controlled, biphasic) Digitimer DS4 +/.44V (analog controlled, biphasic) StimJim (both chs, voltage, in series) +/.13.7V (program controlled, biphasic) StimJim (each channel, voltage) +/.13.7V (program controlled, biphasic) StimJim (both chs, current, in series) +/.13.7V (program controlled, biphasic) StimJim (both chs, current, in series) +/.13.7V (program controlled, biphasic) Intan RHS2116 chip (current) -10.7 to +3.3V (14Vp2p, program controlled, biphasic) As you can see, the StimJim compliance voltage is much lower than most, meaning it cannot be used for higher resistance metal or theta glass stimulating electodes, only for the medium and lower resistance metal electrodes. OK Cancel He	One channel of a StimJim has a compliance voltage of +/- 13.7V, so this one channel can pass a maximum of 137 uA through a 100 Kohm electrode. If the two output channels of one StimJim are put in series, the compliance voltage is not +/-27.4V as the bioRxiv documentation states, but still only +/-13.7V. This can still pass only 137 uA through a 100 Kohm electrode. To determine the resistance of your stimulating electrode and the maximim current this electrode can pass, use either the 'Measure Maximum Current & Electrode Resistance', or gradually increase amplitude of triggered pulses until Compliance Voltage is reached.

Fig. 2.6.1. Choosing the number of channels at startup. Also, information on compliance voltage which determines the maximum amount of current your stimulating electrode can pass is included.

2.7 Setting the StimJim Isolator Layout

After setting the number of channels, the next step is to choose the StimJim Isolator layout (left side of Fig. 2.7.1). If you are using Ch0 only, you can choose whether the various panels appear in the Pulses, Log or Train tabsheets, or appear inline, visible all at once (Fig. 2.7.2). If you are using two channels, you can also choose whether the Ch1 panels are on the right of Ch0 (Fig. 2.4.1), or below Ch0 (Fig. 2.7.3).

The various panels to show (Fig. 2.7.2) include:

- 1) Evoke Single Pulse panel
- 2) Enable Triggering of Constant Amp panel
- 3) Enable Incrementing panel
- 4) Evoke Single Train panel
- 5) Measure Maximum Current and Electrode Resistance panel, and
- 6) Log panel

and can be chosen by clicking the 'View' check boxes on the left side of Fig. 2.7.1.

If you are just viewing one StimJim Isolator program using 1 channel on a standard 1920 width x 1080 height pixel screen, you can fit everything in using inline (Fig. 2.7.2A). If you are viewing two or more StimJim Isolator programs on a standard 1920 width x 1080 height pixel screen, you may wish to use the tabsheets (Fig. 2.7.2B).

If you are viewing one StimJim Isolator program using two channels on a larger 2560 width x 1440 height pixel screen, you may wish to use inline (Fig. 2.7.3A) and only view those essential panels (like 'Enable Triggering of Constant Amp', 'Enable Incrementing' and 'Log' panels). If you are viewing one StimJim Isolator program using two channels on a standard 1920 width x 1080 height pixel screen, you may wish to use tabsheets (Fig. 2.7.3B) to show those same essential panels.

etup	
Set Serial Port Number of Channels Layout and Log/Increment	
StimJim Isolator Layout	Print to Log / Control of Incrementing
Show Tabs or Inline Panels Tabs Inline Panels Show Ch1 Position On the Right Below	Printing Pulses to Log ALL Pulses are Printed to Log (default) (Except for Pulses with Periods < 55 ms) NO Pulses are Printed to Log (Except when Compliance Voltage is Reached and During Incrementing)
View View Current/Voltage and Duration PulseFields Evoke Single Train Measure Current and Electrode Resistance Log If this is long only it	Use Interval > Longest Pulse Period in Train (This affects Incrementing with two or more pulses) Use Interval is Greater than Longest Pulse Period in Train is chosen, and when the interval set in] Interval (ms) > PulsePer in Train ger than the longest pulse period in the train (and shorter than the intertrain period, then the Current and Electrode Resistance of the LAST pulse of a train, will be measured and
Save Log Files into Folder And w C:\users\Public\Documents\StimJimIso\StimJimIso1\	when Incrmenting with two or more pulses, such as with paired pulses, or trains with ent number of pulses, this pulse group would be treated as 1 pulse for Incrementing.
	OK Cancel Help

Fig. 2.7.1. Setting the StimJim Isolator layout and printing to the Log. The upper left panel chooses the StimJim layout, the lower left side and upper right panel chooses whether all pulses are printed to the Log, and the lower right panels control incrementing.

\mathbf{A}		I K	✓ SJI SjDefault — □ X	✓ SJI SjDefault — □ X	🔻 SJI SjDefault — 🗆 🗙
	Channel 0		Pulses Log Trains Ch 0 🚍	Pulses Log Trains Ch 0 🚍	Pulses Log Trains Ch 0 =
	Evoke Single Pulse		Evoke Single Pulse	Measure Maximum Current & Electrode Resistance	Evoke Single Train
			Enable Triggering of		10 Pulse Period (ms)
	Enable Triggering of Constant Amp Pulses		Constant Amp Pulses	800 RequestCurrent (uA) Warning	1000 Train Duration (ms)
	Current or Voltage Current (uA) ~		Current or Voltage Current (uA) ~	Time/Inc# uA uA A% Kohm	
	20 Pulse Amplitude (uA)		20 Pulse Amplitude (uA)	13:24:22 Enable Ch0 ConstantAm	
	20 Manual Increment (uA)		20 Manual Increment (uA)	13:24:41 -150 -148 -1% ??K 13:24:43 -150 -148 -1% ??K	
	Polarity 🦾 💶 🖓 տես		Polarity J, c	13:24:45 -150 -148 -1% ??K	
	100 Pulse (Phase0) Duration (us)		100 Pulse (Phase0) Duration (us)	13:24:46 Enable Ch0 Increment	
<	0 Inter-Phase1 Duration (us)		0 Inter Phase1 Duration (us)	13:24:47 Start Next Increment	
	2 Phase2 Dur (x0-x4) 200us		2 Phase2 Dur (x0-x4) 200us	2 -100 -97 -3% ??K 3 -150 -148 -1% ??K	
	Enable Incrementing			4 -200 -197 -2% ??K 5 -250 -247 -1% ??K	
	1 Number of Repeats		Enable incrementing	6 -300 -296 -1% ??K 7 -350 -346 -1% ??K	
	1 Number of Holds at One Amp		1 Number of Repeats	8 -400 -396 -1% ??K	
	20 Starting Pulse Amplitude (uA)		1 Number of Holds at One Amp	13:25:01 Resume Ch0 ConstantAm	
	20 Auto Increment (uA)		20. Starting Bulso Amplitude (uA)	13:25:03 -150 -148 -1% ??K	
	200 Ending Pulse Amplitude (uA)		20 Starting Pulse Amplitude (uA)	13:25:05 -150 -148 -1% ??K	
	10 Number of Increments		20 Auto Increment (uA)	12:25:08 Steened Ch0	
			200 Ending Pulse Amplitude (uA)	13:25:08 Scopped Cho	
	Evoke Single Train		10 Number of Increments	< >	
	10 Pulse Period (ms)				
	1000 Train Duration (ms)		stopped	Stopped	stopped
	100 Number of Pulses		0 Max Cur (uA) 0 Re (KO) C	0 Max Cur (uA) 0 Re (KO) C	0 Max Cur (uA) 0 Re (KO) C
	Measure Maximum Current & Electrode Resistance				
	800 RequestCurrent (uA) Warning				
	Time/Inc# Reg Sent A% Re				
	19:06:50 Enable Ch0 ConstantAm				
	19:06:55 -20 -15 -25% ??K 19:07:00 -20 -14 -30% ??K				
	19:07:03 Enable Ch0 Increment				
	1 -20 -14 -30% ??K				
	2 -40 -2 -95% 6850 3 -60 0 -100% ?? ✓				
	< >				
	Stopped				
	0 Max Cur (uA) 0 Re (KΩ) C				

Fig. 2.7.2. Ch0 only layout showing all normal panels A) Inline, and B) in Pulses, Log and Train tabsheets.





Fig. 2.7.3. Two channel layout. A) Inline Above/Below configuration on a 2560 x 1440 pixel screen. B) Tabsheet Above/Below configuration on a 1920 x 1080 pixel screen. Ch0 shows the Pulses tabsheet, and Ch1 shows the Log tabsheet.

Furthermore, once you have determined the stimulus pulse current or voltage mode, whether the pulse is monophasic, biphasic, or triphasic, and the pulse durations, you can uncheck the **'Current/Voltage and Duration PulseFields'** checkbox and only show the amplitude related pulse fields (**'Pulse Amplitude'**, **'Manual Increment'** and **'Polarity'** pulse fields (see Fig. 3.1.2.1).

2.8 Printing to the Log

The lower left side of the 'Layout and Log/Increment' tabsheet ($\underline{Fig. 2.7.1}$) shows the 'Save Files to Folder' checkbox which controls whether the Log files will be saved at the end of the experiment when the StimJim Isolator program is shut down. The panel beneath shows in what folder the Log files are saved to.

The upper right panel of the 'Layout and Log/Increment' tabsheet (Fig. 2.7.1) shows how to control printing oulses to the Log. When the top radiobutton is selected, ALL pulse TimeOfDay, Requested amplitude, Sent amplitude, the change in amplitude are printed to the Log, and the approximate resistance if the compliant voltage is reached (ie. Δ % is below -10%) is also printed.

When the lower radiobutton is selected, NO pulse values are printed to the Log except when the compliance voltage is reached.

CHAPTER 3 – Running the StimJim Isolator Program

3.1 Set Stimulus Pulse Parameters

Now it's time to play around with the StimJim Isolator program to see how it works.

3.1.1 Setting Up the Test Recording Configuration

In experimental conditions, the stimulating electrode is directly connected to the + and – terminals of each StimJim channel, with obviously no connection to ground (Fig. 3.1.1.1A). The easiest way to record from the StimJim for testing its output is to set the Current/Voltage Mode to Voltage Mode and directly plug in the data acquisition input with the negative terminal grounded (Fig. 3.1.1B). However, in this way you won't be able to record reaching maximum voltage of ± 15 volts, only ± 10 volts. The configuration that most closely mimics the experimental configuration is to stimulate in Current Mode and set up a voltage divider and measure the voltage drop across a 1 Kohm resistor (and therefore the current passing through it). And the total resistance should your electrode resistance, in this case 1K + 99K = 100Kohms (Fig. 3.2.1C). By using a voltage divider, you will be able to record reaching the compliance voltage of ± 13.7 volts. Both independent Chs 0 and 1 have a compliance voltage of approximately ± 13.7 volts (but see Section 3.6), so each channel can separately pass a maximum of 137 uA through a 100 Kohm electrode.



Fig. 3.1.1.1. Configurations for A) stimulating in an experiment, B recording in Constant Voltage mode, and C) recording in Constant Current Mode using a voltage divider. Measurement of current is from the voltage drop across a 1 Kohm resistor, and therefore, measurement of 1 mV equals 1 uA.

A voltage divider can easily be constructed using two BNC to double banana connectors, an allegator clip, and resistors (Fig. 3.1.1.2).



Fig. 3.1.1.2. Two voltage dividers connected to the two StimJim outputs.

3.1.2 Control of Pulse Amplitude

The values in the Constant Amplitude panel are the pulse value

s that the Evoke Single Pulse will immediately output when that button is clicked, or when the Enable Triggering of Constant Amplitude Pulses is clicked when then triggered by a pulse to Input 0 or input1 from the data acquisition board.

First of all, select constant current or constant voltage (default is constant current). To my knowledge, most extracellular stimulation uses constant current because the amount of current delivered will theoretically not change if the resistance of the stimulating electrode changes (for whatever reason including electrode polarization).

Then set the '**Pulse Amplitude**' value - the amplitude of the initial phase of the pulse. Also set the '**Manual Increment**' value (this is the amount the pulse amplitude will change when the Pulse Amplitude is manually incremented or decremented). Manually incrementing the pulse amplitude is done by placing the cursor on the '**Pulse Amplitude**' field and clicking the LEFT mouse button. Decrementing the pulse amplitude is done by clicking the RIGHT mouse button.





Fig. 3.1.2.1. Set stimulus pulse parameters. A) After the current/voltage and pulse phase durations are decided on, you can uncheck the '**View -> Current/Voltage and Duration PulseFields**' (left side <u>Fig. 2.7.1</u>) which B) shows only the pulse amplitude fields that are likely needed to be changed during an experiment. The current/voltage and pulse phase duration fields are no longer shown.

Then set the **'Polarity'** value. Since extracellular stimulation of axons is achieved by driving the electrode negative, for monopolar and bipolar concentric electrodes, the usual polarity would be negative. However, if you are using a side-by-side bipolar electrode, which polarity to use would be which polarity experimentally caused the larger PSP.

3.1.3 Control of Pulse Phase Durations

The '**Pulse (Phase0) Duration (us)**' field controls the duration of the initial phase of the pulse stimulation. This is the one that should activate axonal firing.



Fig. 3.1.3.1, Different types of pulses output by StimJim. A Monophasic pulse of 200uA and 500us duration. B1) Biphasic pulse with second phase of same duration and amplitude, but opposite polarity. B2) Biphasic pulse with second phase of twice duration, half amplitude, and opposite polarity. B3) Biphasic pulse with second phase of four-times duration, quarter amplitude, and opposite polarity. C1, C2, C2) Triphasic Pulse. Same biphasic pulses as B1, B2 and B3 except an interphase gap is placed between the first and second phases with duration set by the 'Inter-Phase1 Duration (us)' field, and of zero amplitude. The less than rectangular pulse shape is due to the 25us WinLTP sample interval.

The **'Phase2 Duration (0x-4x Phase0)'** field is the duration of Phase0 from x0 = no Phase2, ie a monophasic pulse, (Fig. 3.1.3.1A). x1 biphasic stimulation (Phase2 is equal in duration and amplitude but of opposite polarity (Fig. 3.1.3.1B1). x2 biphasic stimulation (Phase2 is twice duration and half amplitude of Phase0 (Fig. 3.1.3.1B2). x4 biphasic stimulation (Phase2 is four times duration and one-fourth amplitude of Phase0 (Fig. 3.1.3.1B3). Phase2 has the same amount of charge delivered as Phase0 (but of opposite polarity), but its amplitude can be less therefore ensuring that it is Phase0 and not Phase2 that stimulates the axons.

The 'Inter-Phase1 Duration (us)' field sets the gap between Phase0 and Phase2, and is between 0us (no gap) and 2000us that some researchers like to put in (Fig. 3.1.3.1C).

Once you have set which Current / Voltage Mode, the monophasic / biphasic / triphasic pulse shape, and set the Phase0, InterPhase1 and Phase2 durations, you really don't need to constantly view those fields any longer. To stop viewing them, you can uncheck the **'View -> Current/Voltage and Duration PulseFields'** (left side <u>Fig. 2.7.1</u>) which B) and then show only the pulse amplitude fields (<u>Fig. 3.1.2.1</u>B).

3.2 Evoke Single Pulses

To immediately evoke a single pulse, click the **'Evoke Single Pulse'** button (see the top panels in <u>Fig. 2.4.1</u>). The evoked pulses will use the pulse values in the Constant Amplitude panel.

3.3 Enabling Triggering of Constant Amplitude Pulses

Clicking the Ch0 and/or Ch1 'Enable Triggering of Constant Amplitude Pulses' button starts the StimJim to output a Ch0 and/or Ch1 monophasic, biphasic or triphasic pulse for each Input0 and/or Input1 pulse (Fig. 3.3.1). If the 'Enable Triggering of Constant Amp Pulses' button is clicked ON the Constant Amp panel changes to GREEN, and all other panels go to gray.



Fig. 3.3.1. Constant Amplitude stimulation. The top left trace shows Ch0 50 ms paired pulse stimulation. Only the last pulse amplitude of a paired pulse reading is printed to the Ch0 Log. The second left trace shows Ch1 100 ms paired pulse stimulation. Two pulse amplitude readings are printed to the Ch1 Log, one for each pulse. The top trace also shows a LTP induction train (100 pulses at 10 ms pulse periods) and it only outputs the single last pulse amplitude reading to the Ch0 Log. (The StimJim / StimJim_Isolator can follow trains to at least 2000 Hz.) Similarly the second trace shows Ch1 3 theta burst stimulation of 5 pulses/burst and 10 ms pulse periods. Just the last pulse amplitude3 theta burst readings are printed to the Ch1 Log.

A Triggering 2 Pulses at 55 ms Intervals

Pulse 1Started T train with parameters of PulseTrain 0Pulse 2Started T train with parameters of PulseTrain 0Train complete. Delivered 1 pulses.
Current/Voltage by stage:
Ch0Ch1Stage 0-95uA,
Stage 10uA,
0uA,

B Triggering 2 Pulses at 60 ms Intervals

Pulse 1	Ise 1 Started T train with parameters of Pulse					
	Train complete. Delivered 1 pulses.					
	Current/Voltage by	stage:				
	Ch0	Ch1				
	Stage 0 -95uA,	0uA,				
	Stage 1 97uA,	0uA,				
Pulse 2	Started T train wit Train complete. Del	h parameters of PulseTrain	0			
Pulse 2	Started T train wit Train complete. Del Current/Voltage by	h parameters of PulseTrain ivered 1 pulses. stage:	0			
Pulse 2	Started T train wit Train complete. Del Current/Voltage by Ch0	h parameters of PulseTrain ivered 1 pulses. stage: Ch1	0			
Pulse 2	Started T train wit Train complete. Del Current/Voltage by Ch0 Stage 0 -95uA,	h parameters of PulseTrain ivered 1 pulses. stage: Ch1 OuA,	0			

Fig. 3.3.2. The StimJim output in Cermak's stimPulserClientBA.exe when triggering A) 2 pulses at 55 ms or shorter intervals, and B) 2 pulses at 60 ms or longer intervals. Note that the second pulses have the same 6 lines and full stimulation information, wheras the first pulse at 55 ms interval has only one line and no stimulation information, but the first pulse at 60 ms interval has the full 6 lines with full stimulation information.

If '**NO Pulses are Printed to Log**' is chosen (right side <u>Fig. 2.7.1</u>), no pulse amplitudes will be printed to the Log during Constant Amplitude stimulation, except if the compliance voltage is reached (and during incrementing). If '**ALL Pulses are Printed to Log**' is chosen, all pulse amplitudes will be printed to the Log during Constant Amplitude stimulation.

The one exception to this is when pulse periods are 55 ms or less. The reason for this is that because the StimJim output when triggering 2 pulses at 55 ms or lower intervals, the second pulse has 6 lines and full stimulation information, whereas the first pulse has only 1 line and no stimulation information (Fig. 3.3.2A). The StimJim output when triggering 2 pulses at 60 ms or higher intervals, both the first and second pulses have 6 lines and full stimulation information (Fig. 3.3.2B).

3.4 Auto Incrementing Pulse Amplitude

3.4.1 Incrementing with One Pulse per Sweep

Incrementing every pulse (ie. one pulse/sweep) is straightforward. Incrementing can take place at least every 150 ms.

When the **'Enable Triggering of Incrementing Amplitude Pulses'** button is clicked, this begins the auto incrementing of pulse amplitude values beginning with the **'Starting Pulse Amplitude'** value, incrementing each pulse by the **'Auto Increment'** value, and ending with **up to** the **'Ending Pulse Amplitude'** value (Fig. 3.4.1.1). The **'Number of Increment Steps'** is a read only field. This incrementing will repeat X **'Number of Repeats'**. For incrementing every pulse, the **'Number of Holds at One Amplitude'** must be 1.

If the '**Enable Incrementing**' button is clicked ON the auto-incrementing panel changes to GREEN, and all other panels go to gray (Fig. 3.4.1.2A2).

Enable Incrementing				
1	Number of Repeats			
1	Number of Holds at One Amp			
20	Starting Pulse Amplitude (uA)			
20	Auto Increment (uA)			
200	Ending Pulse Amplitude (uA)			
10	Number of Increments			

Fig. 3.4.1.1. Auto-incrementing fields.

Figs. 3.4.1.2A1 and B shows that clicking the 'Enable Triggering of Constant Amp Pulses' button (arrow) produces no pulses until the triggering pulses from the data acquisition board arrives. Just a single click on the 'Enable Incrementing' button (Fig. 3.4.1.2A2 arrow) starts the auto-increment sequence of 8 'Number of Increments' that occurs one time. After the 8th 'End Pulse Amplitude' is reached, the program (Fig. 3.4.1.2A3) automatically goes back to the 'Enable Triggering of Constant Amp Pulses' sequence. If the 'Stop' button is pressed (Fig. 3.4.1.2A4. arrow), all Constant Amp and Increment Amp pulses stop even if triggering pulses from the data acquisition board continues.



Fig. 3.4.1.2. Demonstration of triggering the A1) Constant Amp, A2) the Incrementing Amp sequence, A3) the automatic return to Constant Amp when then incrementing finishes, and A4) the **'Stop'** button which stops all pulse output even though the triggering pulses from the data acquisition board continues.

In Fig. 3.4.1.2, the incrementing was allowed to go to completion with 8 increment steps. In Fig. 3.4.1.3, the incrementing is prematurely stopped after 5 increment steps by clicking the **'Enable Triggering of Constant Amp Pulses' button** a second time. This is useful in doing I/O curves where the maximum amount you want to stimulate is less than the **'Ending Pulse Amplitude'**. You can also change the **'Ending Pulse Amplitude'** field while incrementing.

If you increase the '**Number of Repeats'** field to above one, this causes repeating of the increment sequence (Fig. 3.4.1.4).

One of the major improvements of StimJim Isolator 1.00 over the beta 0.12 version is the ability to increment Ch0 and Ch1 at the same time as shown in Fig. 3.4.1.5.

If you are running 1 pulse/sweep, when you set the '**Number of Holds at One Amplitude**' to greater than one (<u>Fig. 3.4.1.6</u>), you can either:

- a) Do signal averaging with the Number of Hold pulses, or
- b) Increment sweeps with the Number of Hold pulses in each sweep.



Fig. 3.4.1.3. Same as Fig. 3.4.1.2B except that the 'Enable Triggering of Constant Amp Pulses' is clicked after 5 increments to prematurely stop incrementing and go back to Constant Amp pulses.



Fig. 3.4.1.4. The '**Number of Repeats**' field has been increased to 3 to produce 3 repeat increments which automatically finishes and goes back to Constant Pulses.



Fig. 3.4.1.5. Incrementing Ch0 and Ch1 simultaneously. Incrementing Ch1 starts 4 pulses after starting incrementing Ch0.



Fig. 3.4.1.6. A) Incrementing every third pulse with the '**Number of Holds at One Amplitude**' value set to 3. B) Log view of the incrementing from 50 to 400 uA in steps of 3.

3.4.2 Incrementing with Two or More Pulses per Sweep – Using Hold

Protocols with incrementing with two or more pulses/sweep are a bit more difficult than with 1 pulse/sweep. It is strongly advised that you test out these protocols **BEFORE** you start your experiment. You may need to use 'Interval Value is > Pulse Period in Train', <u>Section 3.4.3</u>.

One of the major improvements of StimJim Isolator 1.00 over the beta 0.12 version is the ability to increment sweeps with more than one pulse. If you were incrementing every 4 paired-pulses with say 100 ms pulse period, that would mean incrementing every 8 pulse. The '**Number of Holds at One Amplitude**' in this case would therefore be Holds = 8 (red arrow, Fig. 3.4.2.1). The paired pulse periods can be as low as 0.5 ms.



Holds = 2 Holds/Paired-Pulse x 4 paired pulses = 8. 100 ms Pulse Period.

Fig. 3.4.2.1. Incrementing Paired-Pulse stimulation every 4^{th} paired pulse. For 100 ms pulse period here, that would be just like incrementing every 8 pulses, so the **Number of Holds at One Amplitude** would be = **8** (red arrow).



Holds = 14 Total, 20 to 2000 ms Pulse Period

100000 ms

Fig. 3.4.2.2. Incrementing Paired-Pulse stimulation for 7 pulse pairs; 20, 50, 100, 200, 500, 1000 and 2000 ms pulse period. For each paired pulse, the Hold is 2 per paired pulse. The total Number of Holds is therefore 14 (red arrow).

Fig. 3.4.2.2 shows another common method of incrementing. In this case there are 7 paired pulses whose pulse period increments by the data acquisition system from 20, 50, 100, 200, 500, 1000 and 2000 ms. And each of this group should then be incrementing from 100 to 200, and to 300 uA by the StimJim. This incrementing is achieved by holding the 20 to 2000 ms paired pulses by 2 Holds/pulse-pair. The total Number of Holds would therefore be 7 paired-pulses x 2 Holds/paired-pulse = 14 Holds (red arrow, Fig. 3.4.2.2).

3.4.3 Using Interval Value is > Pulse Period in Train

In some cases, using the 'Interval > Pulse Period in Train' is necessary to get the correct increment. What it does is when the interval value is greater than the pulse period of one or more pulses, those pulses are considered as one pulse – both in printing to the Log (only the last pulse is printed), and in incrementing.

This interval field (see red arrow in Fig. 3.4.3.1) s made visible by checking the **'Use Interval > Longest Pulse Period in Train'** checkbox in the **'Layout and Log/Increment'** page of the **'Setup'** dialog (lower right side of Fig. 2.7.1).

One situation is where you want to increment groups of pulses with different number of pulses. Setting the **'Number of Holds at One Amplitude'** to the number of pulses in a pulse group will not work because the number of pulses changes.

In Fig. 3.4.3.1, the first group of pulses are 7 paired-pulses with periods of 20, 50, 100, 200, 500, 1000 and 2000 ms, or 14 pules total. This is a perfectly common paired pulse period logarithmic increment. The longest period between paired pulses is the start of each paired-pulse occurring every 4000 ms minus the 20 ms paired-pulse period, or 3980 ms. The second and third groups of pulses are 4 paired-pulses with periods of 50, 100, 500 and 2000 ms, or 8 pulse total, with the longest period between paired pulses of 3950 ms. Using a 5000 ms **'Interval > Pulse Period in Train'** (that is longer than the 3980 ms pulse period) achieves a 100 uA incrementing for each pulse group.



Hold = 1, 5000ms = Interval > Pulse Period in Train

Fig. 3.4.3.1. Incrementing over different number of pulses is achieved using **'Interval > Pulse Period in Train'**. The first group of pulses are 7 paired-pulses with periods from 20 to 2000 ms, or 14 pulses total with the longest period between paired pulses of 3980 ms. The second and third groups of pulses are 4 paired-pulses with periods from 50 to 2000 ms, or 8 pulse total with the longest period between paired pulses of 3980 ms. Using a 5000 ms **'Interval > Pulse Period in Train'** field (red arrow on right) that is longer than the 3980 ms pulse period achieves a 100 uA incrementing for each group.

Another situation is where an **error** occurs during incrementing – particularly when 2 channels are being simultaneously incremented. An example of such an error (ie bug) is shown in Fig. 3.4.3.2A. This figure shows 2 channels of 3 groups of paired-pulses, with each group containing 4 paired-pulses of 200 ms pulse periods, and 2800 ms intergroup intervals. Since the **'Number of Holds at One Amplitude'** is 8 you should expect the first pulse of the 2nd and 3rd groups to be incremented. But the first pulse of the 2nd group is not (red arrow in Fig. 3.4.3.2A). (This is a subtle error and only occurs if the Ch1 **'Enable Incrementing'** button is clicked first, and Ch1 stimulation preceeds Ch0 stimulation.)

The **fix** to this error is to invoke the **'Interval > Pulse Period in Train'** field, and set its value to 300 ms, ie longer than the 200 ms paired-pulse pulse period (red arrow, Fig. 3.4.3.2B). When this is done, it means that the paired-pulse produces one pulse increment, and when the **'Number of Holds at One Amplitude'** is now set to 4, the increment now occurs in the first pulse of the 2nd and 3rd pulse groups.



Fig. 3.4.3.2. ERROR in output in A) is FIXED in B) by using **'Interval is > Pulse Period in Train'** (red arrow). Incrementing Ch0 and Ch1 paired pulses every 4th paired-pulse. Pulse period is 200 ms.

3.5 Finding Maximum Stimulating Current and Approximate Electrode Resistance

As far as I know, the StimJim / StimJim_isolator is one of the few (or the only?) stimulus isolator that is capable of measuring amount of current injected and approximate electrode resistance during an experiment while in the tissue.

To find out the maximum current your electrode can pass for a given compliance voltage, and the approximate electrode resistance, you must pass a constant current pulse that is larger than the StimJim is capable of passing. This will be when the compliance voltage is reached, and the Cermak et al (2019) says it is \pm 13.7 volts for the StimJim. The maximum amount of current an electrode of a given resistance can pass is determined by the compliance voltage (the maximum voltage an isolator can pass in current mode) by this equation:

Imax = Vcompliance / Relectrode

Just because the resistance of your stimulating metal microelectrode says "100 Kohms", that is not really the resistance of your electrode to a 100 us stimulating pulse. Most metal microelectrodes resistances are measured with a 1 KHz sine wave.

As A-M Systems states on their website (<u>https://www.a-msystems.com/p-356-epoxy-insulated-tungsten-microelectrode.aspx</u>):

"Metal electrodes do not behave as resistors due to polarization. They have low series resistances for high frequency signals and high series resistance for low frequency signals. For example, for DC inputs a typical tungsten microelectrode will have a series resistance of about 200 M Ω . However, for a 1 kHz sine input, the same electrode will have an impedance of 1 M Ω . Its impedance will further drop to only 100 k Ω for a 100 µs pulse."

So your "100 Kohm" electrode may only have a 10 Kohm resistance for a 100 us pulse and be able to deliver 1370 uA by one StimJim channel.

The maximum current is determined when the current SENT (and measured) by the StimJim is 10% less than the current REQUESTED by this StimJim_Isolator program. This is known only when the compliance voltage is reached. The electrode resistance can then be calculated when the compliance voltage is reached and the maximum current is known by the equation:

Relectrode = Vcompliance / Imax

Clicking the 'Measure Maximum Current & Electrode Resistance' button will pass the 'Requested Current' amount, not the vale in the 'Pulse Amplitude' field. You can set the 'Requested Current' value to be just a bit more than you will ever want to pass in your experiment (say 600 uA when the maximum stimulation will be 500 uA) and the compliance voltage is not reached (Fig. 3.5.1A), or you can set it to an amount (say 3300 uA, the maximum amount the StimJim can theoretically pass) that will be way above the amount required to pass the compliance voltage (Fig. 3.5.1B).

Note that this measures here the maximum current of -1038 uA that can pass through a 10 Kohm 1% resistor, meaning the compliance voltage is actually -10.38 volts, not the -13.7 volts that Cermak et al (2019) state (see <u>Appendix 1.1</u>).

This same result can be obtained by changing the '**Pulse Amplitude**' value to a high value. The '**Requested Current**' field is only put in for convenience – a quick way to measure maximum current and approximate electrode resistance.

Measure Maximum Current & Electrode Resistance	B Measure Maximum Current & Electrode Resistance
600 RequestCurrent (uA) Warning	3300 RequestCurrent (uA) Warning
Time/Inc# Req Sent ∆% Re uA uA ∆% Kohm	Time/Inc# Req Sent ∆% Re uA uA Kohm
Measure Current & Re Evoke Pul 12:58:30 -600 -594 -1% ??K	Measure Current & Re Evoke Pul 13:05:47 -3300 -1038 -69% 13K
< >>	< >>
Stopped	Stopped
0 Max Cur (uA) 0 Re (KΩ) C	-1038 Max Cur (uA) 13 Re (KΩ) C
Req=-100uA Sent=-98uA -2% ??K	Req=-3300uA Sent=-1038uA -69% Ovr

Fig. 3.5.1. Clicking the 'Measure Maximum Current & Electrode Resistance' button will pass the 'Requested Current' amount, not the value in the 'Pulse Amplitude' field. You can set the 'Requested Current' value to be A) just a bit more than you will ever want to pass in your experiment (say 600 uA when the maximum stimulation will be 500 uA) and the compliance voltage is not reached, or you can set it to B) an amount (say 3300 uA the maximum amount the StimJim can theoretically pass) that will be way above the amount required to pass the compliance voltage. The maximum current is in the Log 'Sent uA' column, and the electrode resistance is in the Log 'Re Kohm' column, and are also printed in fields below in red. Note that the resistance is 13 Kohms, 3 Kohms higher than the 10 Kohm resistor used.

Clicking the '**Warning**' button in Fig. 3.5.1 shows online information on measuring maximum current and electrode resistance in Fig. 3.5.2.

We strongly recommend that you do this MaxCurrent/Re measurement with the electrode in the tissue or slice at the end of the experiment because:

- 1) the maximum amount of current the electrode can pass can decrease four-fold with the electrode in the slice compared with in the saline, and
- 2) even the currents generated even by the low 13.7 volts compliance voltage can change or damage the slice, so it is appropriate to do this at the end of the experiment.

We recommend you do this measurement sparingly, one or two pulses, because:

3) if this measurement is done repeatedly, the maximum amount of current the electrode can pass can be halved.

Initially rely on the triggered pulses to show if compliance voltage is reached. And after this MaxCurrent/Re measurement is done in the slice, pull the electrode out of the slice but still be in the saline, and do this MaxCurrent/Re measurement again.

MEASURE MAXIMUM CURRENT AND ELECTRODE RESISTANCE

IMPORTANCE OF COMPLIANCE VOLTAGE

The maximum amount of current an electrode of a given resistance can pass is determined by the compliance voltage (the maximum voltage an isolator can output in the current mode). The equation is:

Imax = Vcompliance / Relectrode

Just because the resistance of your stimulating metal microelectrode says "100 Kohms", that is not necessarily the resistance of your electrode to a 100 us stimulating pulse. Most metal microelectrodes resistances are measured with a 1 KHz sine wave.

As A-M Systems states on their website:

"Metal electrodes do not behave as resistors due to polarization. They have low series resistances for high frequency signals and high series resistance for low frequency signals. For example, for DC inputs a typical tungsten microelectrode will have a series resistance of about 200 M Ω .

However, for a 1 kHz sine input, the same electrode will have an impedance of 1 M Ω . Its impedance will further drop to only 100 k Ω for a 100 µs pulse."

So your "100 Kohm" electrode may only have a 10 Kohm resistance for a 100 us pulse and be able to deliver 1370 uA by one StimJim channel. Run the tests here to see the actual maximum current your electode can deliver for a given compliance voltage, at ca. 100 us pulse duration, and see what its actual resistance is.

HOW THE MAXIMUM CURRENT AND ELECTODE RESISTANCE ARE MEASURED

Maximum current is determined when the current SENT (and measured) by the StimJim is 10% less than the current REQUESTED by this StimJim_Isolator program. This is known only when the compliance voltage is reached. The electode resistance can then be calculated when the compliance voltage is reached and the maximum current is known by the equation:

Relectrode = Vcompliance / Imax

WARNING: MEASURE THIS WITH THE ELECTRODE IN THE TISSUE OR SLICE AT THE END OF AN EXPERIMENT, AND DO THIS MEASUREMENT SPARINGLY

We strongly recommend that you do this MaxCurrent/Re measurement with the electode in the tissue or slice at the end of the experiment because:

1) the maximum amount of current the electrode can pass can decrease four-fold with the electrode in the slice compared with in the saline, and

2) even the currents generated even by the low 13.7 volts compliance voltage can change or damage the slice, so it is appropriate to do this at the end of the experiment.

We recommend you do this measurement sparingly, one or two pulses, because:

3) if this measurement is done repeatedly, the maximum amount of current the electode can pass can be halved.

Initially rely on the triggered pulses to show if compliance voltage is reached. And after this MaxCurrent/Re measurement is done in the slice, pull the electrode out of the slice but still in the saline, and do this MaxCurrent/Re measurement again.

OK

Fig. 3.5.2. Clicking the '**Warning**' button will bring up the dialog box information on measuring maximum passable current and electrode resistance.

3.6 Calibrating to Find the Real Electrode Resistance and Compliance Voltage

Note that this calibration of your StimJim is **OPTIONAL**, and only affects the accuracy of your resistance readings. What really counts is the current being passed and whether the maximum passable current has been reached (when the compliance voltage has been reached). Since the StimJim Isolator program continually monitors when the Maximum Current has been reached (when the Sent Current is 10% less than the Requested Current), knowing the electrode resistance is not essential, only informative.

Throughout this manual I have been stating that the compliance voltage of a StimJim is ± 13.7 volts. This is the value that Cremak et al. state in their 2019 bioRxiv paper (Table 3.6.1). And this value has been used by the the StimJim_Isolator program to calculate approximate electrode resistance.

In fact, you can determine the actual or true compliance voltage by calibrating your StimJim by putting different resistors of 1% accuracy across the + and – outout terminals of a StimJim channel and have the StimJim measure the maximum current that reached is.

When I did this on my development StimJim (a Labmaker, version 0.18, model bought in June 2021), I found that compliance voltage tends towards -13.7 volts for high resistance resistors (50 - 500 Kohm), but is somewhat, or drastically, reduced for resistors with resistances below that (Table 3.6.2). At 10 Kohm resistance the compliance voltage is only -10.22 volts, and at 2 Kohm it is only -5.27 volts.

This means that the StimJim_Isolator resistance calculations are indeed only approximate. In fact, for my StimJim, the difference between the resistance calculated by the StimJim_Isolator program and the actual 1% resistors are 3 Kohms larger at resistors of 10 Kohms and below (a fairly substantial difference), and only 3-5 Kohms for resistors 20-200K (a fairly insignificant difference) (Table 3.6.3). So for my StimJim, readings of 24 Kohms and above I would take the StimJim_Isolator reading, and subtract 3 Kohms from the actual reading to get a better approximation of true electrode resistance.

The current the StimJim measures can just be 1% resistors across the + and – output terminals (Fig. 3.1.1.1A), or if you also wanted to measure the actual output by measure the voltage drop across a 1 Kohm resistor (Fig. 3.1.1.1C).

Compliand			Calculated	
Voltage	Resistor		Max Current	
(V)		(Kohm)		(uA)
-13.7	/	2	=	-6850 ¹
-13.7	/	4	=	-3425 ¹
-13.7	/	5	=	-2740
-13.7	/	10	=	-1370
-13.7	/	20	=	-685
-13.7	/	50	=	-274
-13.7	/	100	=	-137
-13.7	/	200	=	-68.5
-13.7	1	500	=	-27.4

Table 3.6.1. Calculating the theoretical Maximum Current using a compliance voltage of -13.7 volts and resistances between 2 and 500 Kohms. $V_{compliance} / R = I_{max}$. 1. In Cermak et al's 2019 bioRxiv paper they state that the maximum current output by the StimJim is 3300 uA.

		Max Curr	ent	
		Measure	d by	True
Actual 1	%	and Sen	Compliance	
Resisto	r	StimJin	า	Voltage
(Kohm)		(uA)		(V)
2	*	-2636	=	-5.27
4	*	-1903	=	-7.61
5	*	-1671	=	-8.36
10	*	-1022	=	-10.22
20	*	-580	=	-11.60
50	*	-259	=	-12.95
100	*	-129	=	-12.90
200	*	-66	=	-13.20
500	*	-26.5	=	-13.25

Table 3.6.2. Measuring the Maximum Current sent by the StimJim across an actual resistor of accuracy to 1%. R * $I_{max} = V_{compliance}$. At 50 - 500 Kohms the true compliance voltage is similar ri the 13.7 volts Cermak value, but is quite a bit less below those resistances.

Resistance	е				
Calculated by			Difference		
StimJim		Actual 1%	in	Resistanc	е
Isolator		Resistor	(Cal	ced – Acti	ual)
(Kohm)		(Kohm)		(Kohm)	
5	-	2	=	3	
7	-	4	=	3	
8	-	5	=	3	
13	-	10	=	3	
24	-	20	=	4	
53	-	50	=	3	
105	-	100	=	5	
203	-	200	=	3	
516	-	500	=	16	

Table 3.6.3. The difference between the resistance calculated by the StimJm_Isolator program are usually about 3 Kohms larger.

3.7 Evoke a Single Train

Clicking the 'Evoke Single Train' button will immediately evoke a single train (Fig. 3.7.1).

WARNING: An Input1 trigger pulse can terminate both a Ch0 and a Ch1 Evoked Train (see Appendix 2.1).

Note that programs like WinLTP generate all the digital output needed to generate trains and actually do not need the '**Evoke a Single Train**' capability. This was put in for those data acquisition programs that either do not have the capability of generating trains, or can do so with great difficulty.



Fig. 3.7.1. The **'Evoke Single Train'** button in the Trains tab. Also present are fields to control pulse period, train duration, and the number of pulses

3.8 Saving Settings using Protocol Files

To initially save your values as a protocol file choose the **'Save Protocol File As...'** menu item (see <u>Fig.</u> <u>2.4.3</u>). To resave new values use the **'Save Protocol File'** menu item.

When the StimJim_Isolator program is restarted, the last protocol file saved will be automatically be reloaded.

If you wish to load a different protocol file and values, choose the 'Open Protocol File...' menu item.

CHAPTER 4 – Recording of StimJim Stimulation Using Different Electrodes

4.1 Summary

The object in this chapter was to test and find stimulating electrodes that you can use with the StimJim. Basically, I was looking for electrodes that could output 500 uA during a stimulation pulse. What I found with the electrodes tested was that they could reliably output only 200-300 uA. There are several things to keep in mind:

- 1. These tests are very preliminary usually 1 to sometimes 3 electrodes of each type and they are meant to be just a rough guideline as to what you can use. (Hopefully, more StimJim users can send me information on their stimulating electrodes!)
- It's the low compliance voltage and necessary maximum amount of current needed which will determine what electrodes can be used with your StimJim. The low compliance voltage of my Labmaker StimJim (ver. 0.18, bought June, 2021) is approximately <u>+</u>13.7 volts (and not <u>+</u>27.4 volts) (see Sections <u>1.2</u>, <u>2.6</u>, <u>3.5</u> and <u>3.6</u>, and <u>Appendix 1.1</u>). This is similar to the -10.7 to +3.3 volts of the Itan RHS2116 chip,
 - but much lower than the other isolators with a compliance voltage of <u>+44</u> to <u>+100</u> volts (<u>Fig. 2.6.1</u>), NPI Iso-Stim-II

WPI A365 Digitimer DS3 Multichannel Systems STG-400x A-M Systems 2200 A-M Systems 2300 Digitimer DS4

For slice work, the range I found on an informal poll was 50 to 500uA maximum current needed to be passed. So I was looking for electrodes that could pass at least 500uA with the StimJim. In reality, they could only reliably deliver 200-300uA.

The stimulating electrodes I would tentatively recomend are:

- a) 50um Platinum/Iridium Bipolar Twisted Wire Electrodes, and
- b) Haer 30202 or 30213 Bipolar Concentric, 25um Cone Tip, Platinum/Iridium Electrodes
- 3. As far as I know, the StimJim is the only stimulus isolator that reports the actual stimulus current delivered during a pulse when the electrode is **both in the saline bath and in the tissue**. (One possible exception could be the Intan RHS2116).
- 4) This has led to two important findings:
 - a) The resistance of a new electrode could double when either passing high currents in ACSF, or when doing so in normal (low) current stimulations in a slice, when using biphasic current pulses. This is particularly true for the higher resistance Haer 30203 and 30250 electrodes. Resistance increase is possibly due to 'coating' of the electrode, and using the StimJim biphasic pulses doesn't seem to mitigate the resistance increase all that much (but probably still better than using monophasic pulses).
 - b) When the stimulating electrode in the saline was pushed into the slice, the resistance could sometimes double or even quadruple (like going from 16 to 69 Kohms). This result was suprising, because I had thought the extracellular resistance was only a couple of Kohms.

This was found for the NiChrome bipolar electrode and the Pt/Id monopolar electrode, but the increase was less for the Haer bipolar concentric 30203, and 30202 & 30213 electrodes.

With these very preliminary results, I think the health of the slice is very important, **AND therefore how far you have to push the stimulating electrode into the slice** to get a good stimulus response is therefore also important. With a Haer 30202 electrode, the resistance could almost double by pushing the electrode further into the slice.

Here are the results from our tests.

				Tip Area Relative to	Expected Resistance in	Electrode Stir	e in Saline nJim	Electrode Stir	e in Tissue nJim	Tissue
Manufacturer/Code	Metal	Diameter um	Tip Shape	Haer 30202s 25u cone	Saline Relative To Haer 20202	Resistance Kohm	MaxCurrent uA	Resistance Kohm	MaxCurrent uA	Resistance Kohm
Monopolar Single Wire Custom	Pt/Id	50	Flat	2	6.5K	10K	1084	26K	470	16K
Bipolar Twisted Wire Custom Custom	Pt/Id NiCr	50 50	Flat Flat	4 4	3.25K 	22K 16K	556 712	21K 69K	564 191	0K 53K
Bipolar Concentric Haer 30203 " 30203 Haer 30202 & 30213	Pt/ld Pt/ld Pt/ld	12.5 12.5 25	Cone Cone Cone	0.25 0.25 1	52K 52K 13K	23K 13K	526 859	22K 77K 67K	552 170 196	0K 54K
Bipolar Side-by-Side Haer 30250 " 30250 " 30250	Pt/ld Pt/ld Pt/ld					 		25K 19K 36K	489 617 352	

Table 4.1.1. The current passing ability of a few metal stimulating electrodes, and their approximate resistances. StimJim resistance and maximum current measurements were done with a 100 us pulse, and resistances have been corrected by subtracting 3 Kohms (see Section 3.6). Resistances were taken while the electrode was in the slice and just after it was withdrawn into the saline. These results do not show the sometimes doubling of resistance that could occur with substantial stimulation.

I would like to thank Dr. Patrick Tidball of the Univ. of Toronto for the detailed investigation of the Haer 20203 and 30250 electrodes, Dr. Clair Booth of the Univ. of Bristol for helping me with measurements of the Haer 30203 electrode, Dr. Matt Claydon of the Univ. of Bristol for helping me with measurements of the Haer 30202 electrode, and Drs. Daniel Whitcomb and Alanoud Alqahtani of the Univ. of Bristol for helping me with measurements of the Inter and Pt/Id bipolar twisted wire electrodes, and the Pt/Id monopolar wire electrode.

The high resistance Haer 30203 and 30250 were well summarized by Patrick Tidball:

I initially thought this increase in resistance could be due to electrode polarisation (even though you are using biphasic pulses for the electrode test). But this doesn't seem to be the case because resistance didn't drop back down when I shorted the poles (by touching the connector pins together). However, I was able to 'regain' some current (lower the resistance) by brushing the tips of the electrode gently with a fine paintbrush, though it wasn't a full recovery. So, I'm guessing the resistance increase is due to a 'degradation' (tarnishing or corrosion), or coating.

So, in summary, the situation isn't too bad. When the electrodes are in 'good' condition, they can pass a decent amount of current with the StimJim (~500 - 600uA for the 30203 concentric, and at least 700uA for the 30250 matrix electrodes... possibly as high as 1000uA or more). However, the resistance can increase considerably if the electrodes get 'worn out' after passing high current in saline or slice (but they can be restored to some extent by brushing).

4.2 50um Platinum/Iridium Bipolar Twisted Wire Electrode

50um Pt/Id bipolar twisted wire electrodes are a low cost electrode, and are used in the Kerr Scientific Brain Slice and Tissue Recording System.

When the electrode was pulled from the slice into the saline, the resistance was essentially unchanged, 21 to 22 Kohms, and the maximum passable current was also unchanged, 564 to 556 uAs. The reason for the resistance not changing is likely due to a very healthy slice and the electrode just touching the slice.

I/O curves for the CA1 region of a rat hippocampal slice are shown in Fig. 4.2.1. This was a new electrode.



Fig. 4.2.1. I/O curve with the 50um platinum/iridium bipolar twisted wire electrodes.

4.3 50um Platinum/Iridium Monopolar Single Wire Electrode

Extracellular stimulation usually uses bipolar electrodes to localize the stimulation (the tips of both wires close together with side-by-side electrodes, or a small wire tip and a large return outer shield wire with concentric electrodes). In contrast, monopolar electrodes have a smallish wire tip, but the return wire is an ungrounded silver chloride or stainless-steel wire in the bath. In this case the stimulation is presumably not as localized. However, one internationally well-respected lab, W. C. Abraham's lab, has switched to monopolar 50um tungsten wire stimulating electrodes. And at least for student labs, monopolar Pt/ld, or tungsten electrodes would be an easy, cost-effective electrode choice.

When the electrode was pushed into the slice, the resistance increased from 10 to 26 Kohms, and the maximum passable current decreased from 1084 to 470 uAs. I/O curves were not taken. This was a new electrode.

4.4 Haer 30202 Bipolar Concentric, 25um Cone Tip, Platinum/Iridium Electrode

This was a well-used electrode. Only the maximum current and resistance of this electrode was tested in the saline and in the slice from the pre-frontal cortex. I/O curves were not done because it is difficult to record extracellular field potentials in these slices (patch-clamping is usually done).

In saline this electrode shows a low 13 Kohm resistance and a substantial maximum current passing capability of 859uA. However, when this electrode is placed normally in the slice there was a substantial 3-times increase in resistance, and when it is further pushed into the slice the resistance increases to 5-times. This was a well used electrode.

	Maximum	Electrode		
	Current (uA)	Resistance		
		(Kohms)		
Electrode in saline	859	13K		
Electrode normal amount in slice	348	36K		
Electrode pushed further into slice	196	67K		

Table 4.4.1. Measurement of an 'in use' Haer 30202 electrode In saline and in a pre-frontal cortex slice. The electrode resistance was read from the StimJim and corrected by subtracting 3 Kohms from that value (<u>Table 3.6.3</u>).

4.5 Haer 30203 Bipolar Concentric, 12.5um Cone Tip, Platinum/Iridium Electrode

The Haer 30203 was the concentric bipolar electrode with the smallest tip, 12.5 um diameter.

When the electrode was pulled from the slice into the saline, the resistance was essentially unchanged, 22 to 23 Kohms, and the maximum passable current was also unchanged, 552 to 526 uAs. The reason for the resistance not changing is likely due to a very healthy slice and the electrode just touching the slice.

Fig. 4.5.1 shows recordings of rat hippocampal CA1 fields evoked by Haer 30202 stimulation. The stimulation values were -100 to -250 uA. Fig. 4.5.2 shows recordings of CA1 fields in a different rat hippocampal slice and a different 30203 electrode evoked by StimJim stimulation. The stimulation values were -20 to -80 uA.



Fig. 4.5.1. I/O curve from CA1 region of a rat hippocampal slice using a Haer 30202 electrode with a 12.5 um tip diameter. This data was obtained by Dr. Patrick Tidball, University of Toronto.



Fig. 4.5.1. I/O curve from CA1 region of a different rat hippocampal slice using an old Haer 30202 electrode with a 12.5 um tip diameter. This data was obtained with the help of Dr. Clair Booth of the University of Bristol.

4.6 Haer 30250 Bipolar Side-by-Side, Platinum/Iridium Electrode

Haer 30250 bipolar side-by-side Pt/Id electrodes are fairly high resistance electrodes and are (as far as I can tell) essentially the renamed Haer MX21XEP electrodes. (The MX21XEP were actually the electrodes that Patrick Tidball tested, but since they can be no longer purchased, I have used the Haer 30250 name instead.)

Fortunately, Haer gave us the (1 KHz sine wave in saline) resistance measurements for the 30250 and the MX21XEP electrodes as 200-300 and 300-500 Kohms, respectively. This 200-500 Kohm range is roughly 10 times the 23-36 Kohm range measured by the StimJim to a 100 us pulse of a 30250 electrode in saline. This is roughly the same difference A-M Systems thinks should be for a 1KHz sine wave test in saline and with a 100us pulse (Section 3.5).

For I/O curves and stimulus artifacts Haer 30250 bipolar side-by-side Pt/Id electrodes see Figs. 4.8.1, 4.8.2 and 4.8.3.

4.7 50um Nichrome Bipolar Twisted Wire Electrode

50um NiCr bipolar twisted wire electrodes are a very low cost stimulating electrode.

In the rat hippocampal slice, when going from in the slice into the saline, the electrode resistance decreased from 69 to 16 Kohms (a four-fold decrease), and the maximum current passing capability increased from 191 to 712 uAs (<u>Table 4.1.1</u>). This electrode was almost new.

4.8 Comparison of StimJim vs. MultiChannel Systems STG-4002

In his investigation of Haer 30250 bipolar side-by-side electrodes, Patrick Tidball (Univ. of Toronto) also compared the StimJim to the similar Multichannel Systems STG-4002. Both are program-controlled stimulators / stimulus isolators. The main difference is that the STG-4002 is much better at reducing stimulus artifact than the StimJim, but the StimJim is still acceptable.

Fig. 4.8.1 shows recordings from the CA1 region of a P17 rat hippocampal slice using a used Haer 30250 bipolar side-by-side, platinum/iridium electrode. A 20uA biphasic pulse was first delivered from the StimJim. Then the electrode was switched to the MultiChannel Systems STG-4002 without changing electrode position and another 20uA biphasic pulse was then delivered.

Note that the StimJim response (both FV and fEPSP) was slightly smaller than the STG-4002 response. However, the biggest difference was that the stim artefact is much bigger with the StimJim compared to the STG-4002.

Also note that the latency of the response is shorter with the StimJim. (This is because with the STG-4002, a 40µs zero current delay was put in before the main pulse. Multichannel Systems recommend to do this in the STG manual - it gives time for the high-impedance 'gate' to open before delivering the actual stimulus current (otherwise the full current would not be delivered for the full 100µs duration... i.e., there would be a ramp-up / lag). Maybe this is the reason for the slightly smaller response with the StimJim (i.e., it has a slight ramp-up time as the start of the stimulus current).



Fig. 4.8.1. Recordings from the CA1 region of a P17 rat hippocampal slice using a used Haer 30250 bipolar Side-by-side, platinum/iridium electrode. 20uA biphasic pulses were delivered from both the StimJim and the MultiChannel Systems STG-4002 wwithout changing electrode position. Note that the STG-4002 stimulus artifacts (dark blue trace) were much smaller than the StimJim stimulus artifacts even though the fEPSPs evoked by the STG-4002 were larger.

Fig. 4.8.2 shows recordings from the CA1 region of an adult mouse hippocampal slice using a new Haer 30250 bipolar side-by-side, platinum/iridium electrode. 15uA biphasic pulses were delivered from both the MultiChannel Systems STG-4002 and the StimJim. The first sweep recorded some sweeps with the STG-4002 (-100us, +200us biphasic – the +200us was half the amplitude of the -100us). Then the electrode wires were switched to the StimJim (-100us, +200us biphasic). Next the StimJim again but with -100us, 100us gap, +100us biphasic). Finally, switched back to the STG4002 (-100us, +200us biphasic).

The StimJim responses were again smaller than the STG-4002 responses at the same intensity (15uA). And again, the artefact was also again much bigger for the StimJim, even though the fEPSPs were twice as large. Interestingly, the StimJim responses were identical for both types of biphasic stim (with or without gap).



Fig. 4.8.2. Recordings from the CA1 region of an adult mouse hippocampal slice using a new Haer 30250 bipolar Side-by-side, platinum/iridium electrode. 15uA biphasic pulses were delivered from both the MultiChannel Systems STG-4002 and the StimJim. Note that the Stg-4002 stimulus artifacts (light and dark blue traces) were much smaller than the StimJim stimulus artifacts even though the fEPSPs evoked by the STG-4002 were about twice the size of the StimJim fEPSPs. The sweeps were recorded at 40 kHz, and the traces in the figure are 2 kHz filtered in WinLTP, so the actual artefacts are bigger still.

Fig. 4.8.3 shows I/O curves done with first the STG-4002 and then the StimJim on the same slice and electrode as in Fig. 4.8.1,. The same stimulation intensities (10, 20, 30, 40 & 50 μ A (100 μ s) biphasic pulses with the same polarity were used for both the StimJim and STG-4002. As with the responses recorded at 20uA, the StimJim gives a slightly smaller FV and fEPSP for the same stim intensity across the stimulus range. **However, when comparing the FV-to-fEPSP curves, you can see that they overlay perfectly.** So there is nothing strange about the response elicited by the StimJim, its just that it is slightly smaller.



I/O curves - STG4002 vs SimJim (10, 20, 30, 40 & 50 µA intensities)

Fig. 4.8.3. I/O curves done with first the STG-4002 and then the StimJim on the same slice and electrode a used Haer 30250 bipolar Side-by-side, platinum/iridium electrode as Fig. 4.8.1. Note that the STG-4002 and the StimJim Fiber Volley-to-fEPSP curves overlay completely.

APPENDIX 1 Limitations of the StimJim

Appendix 1.1 The compliance voltage in CURRENT mode with two channels in SERIES is only 13.7V, not 27.4V

Note: These tests were done on a StimJim (v0.18, June 2021) made by Labmaker (who only claim 13.7volts compliance voltage), and may not be the same as those on a StimJim made and tested by Cermak and Newman.

If the two output channels of a StimJim are put IN SERIES and are in CURRENT mode (Fig. A.1.1.1), the compliance voltage of one channel, \pm 13.7 volts, should increase to \pm 27.4 volts according to Cermak et al. (2019), and the StimJim should pass 274 uA through a 100 Kohm resistor. Unfortunately, I found that this is not the case.



Fig. A.1.1.1. Constant current channels 0 and 1 connected in series.

Cermak et al. (2019, *BioRxiv*, doi: <u>https://doi.org/10.1101/757716</u>) state: "Its only drawback is that its compliance voltage is lower, which limits the load resistance that Stimjim can drive. For a given resistance R, each Stimjim channel cannot output a **current** larger than 13.4V/R. Thus, Stimjim is not suitable for high-impedance electrodes such as pulled glass electrodes. Note however, that **Stimjim's compliance voltage can be doubled to roughly ±27 V by connecting the two channels in series.**"

Cermak et al further state, as part of Table A.1.1.1:

	StimJim
Compliance voltage	<u>+</u> 15 V (voltage) <u>+</u> 13.7 V ^ь (current)

Table A.1.1.1: . ^b Compliance voltage can be doubled by connecting both output channels in series.

Fig. A.1.1.2 shows that in Current Mode, when the Ch0 and Ch1 are connected in series, the compliance voltage does NOT increase from \pm 13.7 volts to \pm 27.4 volts. Instead in the current recorded, there is a SLOW RISE to peak taking about 1 ms. This means that a 100 us pulse is severely attenuated above the 13.7 compliance voltage currents.

This 13.7V limitation is important because many (most?) researchers extracellularly stimulate with constant **current** because the stimulation through the tissue (and therefore the voltage change across the tissue) is independent of changes in stimulating electrode resistance. Most extracellular stimulus isolators are in the range of $\pm 44V$ (Digitimer DS4) to $\pm 100V$ (NPI Iso-Stim II) (see Fig. 2.6.1). When researchers use high resistance extracellular metal stimulating electrodes (250 Kohms and above for 1 KHz sine wave and 1/10th that resistance for a 100 us pulse) and want to stimulate up to 500 uA, a compliance voltage of ± 13.7 volts would be too little. See also Sections 1.2 and 4.1.

Fig. A.1.1.2 is a '**WARNING**' dialog that is output when you choose Ch0 and Ch1 in series in constant current mode. Any current outputs above the single channel compliance voltage of 13.7V will be attenuated.



When constant current Ch0 and Ch1 are connected in series, when a 100uA current pulse is requested (below the single channel 13V compliance voltage), output is a correct 99uA for a 100us pulse and 100uA for a 1ms pulse.

However, when a +250uA current pulse is requested (almost twice the single channel 13V compliance voltage across a 100 Kohm resistor). the output slowly rises to +239uA for a 1ms pulse, but only rises to a 168uA pulse for a 100us pulse. This slow rise of over 1ms means that a 100us pulse is strongly attenuated and does not at all reflect the current requested.



Fig. A.1.1.2. The '**WARNING**' dialog output when you choose Ch0 and Ch1 in series in constant current mode. Any current outputs above the single channel compliance voltage of \pm 13.7V will be attenuated. You will not get \pm 27V compliance voltage. Requested outputs of +100 uA and +200 uA, and for 100 us and 1 ms are from Cermak's compiled stimPulserClientBA.exe. Measurement of current is from the voltage drop across a 1 Kohm resistor and with a total resistance of 100 Kohms.

Appendix 1.2 If Ch0 and Ch1 are Triggered Simultaneously, Ch0/Input0 Pulse Preceeds the Ch1/Input1 Pulse

When Chs 0 and 1 are both used and when Chs 0 and 1 are triggered simultaneously, the output pulse from Ch0/Input0 will run and finish first, and the Ch1/Input1 pulse starts almost immediately after (Fig. A.1.2.1, red arrow in the red rectangle).

Inotherwords, the two channels are not completely independent.

This also means that timed detection of a Ch1 evoked PSP will be different if Ch0 and Ch1 are triggered at the same time.

To 'fix' this problem, either 1) stagger the triggering of Ch1 by 100 us or more after the Ch0 pulse ends, or 2) use Ch0 of two StimJims (remember that they are very inexpensive).

Note: this is not a bug, it's just a design decision.



Fig. A.1.2.1. The response to 2 ms Ch0 and Ch1 StimJim outputs (100 us PulsePhase0 duration, 100 us InterPhase1 gap, and 400 us PulsePhase2 duration). The less than rectangular pulse shape is due to the 25us WinLTP sample interval. This output was actually controlled by the stimPulserClientBA.exe, but the same results were also obtained with the StimJim_Isolator.exe.

Appendix 2 Bugs in the StimJim / StimJim Isolator Program

Appendix 2.1 Evoked Single Train can be Terminated by an Input Pulse

The inability to turn off an Input1 trigger input. If you are using Ch0 and Ch1 independently, pulses to Input1 will terminate any Ch0 or Ch1 Evoked Single Trains. This will happen once triggering of Ch0 and/or Ch1 has been enabled – even if the enabling has been stopped (Fig. A.2.1.1). So, if you want to evoke Ch0 or CH1 single trains, make sure that there are no incoming pulses to Input1.



Fig. A.2.1.1. If Ch0 and Ch1 are chosen to work independently, and the either the Ch0 or Ch1 **'Train'** tab is clicked, a **'WARNING'** dialog box is output once to state that pulses to Input1 will truncate both Ch0 and CH1 evoked single trains.

Appendix 2.2 Incrementing constant voltage of Chs 0 & 1 in series - the last pulse at +30v goes to -30v



Fig. A.2.2.1. Incrementing from 2 volts to 30 volts. At 30 volts the output becomes a negative 30 volts.

Appendix 3 Examining Grounding Between **Pulses**

When recording in Voltage mode, one can just barely detect the difference between the Ground mode (immediately before and after the triphasic pulse), and the slight negativity during the "0"V inter phase in Fig. A.3.1A, and in the three "0"V phases in Fig. A.3.1.B.



A Slight Negativity During "0"V of a Triphasic -0.1V -> 0V -> 0.1V Pulse

Slight Negativity During All "0"Vs of a Triphasic 0V -> 0V -> 0V Pulse Β



Fig. A.3.1. The difference between Ground mode (before and after the triphasic pulses and during the "0"V phases in the inter phase of A, and in all three phases of B. Twenty sweeps were averaged (blue lines) and the recording of a single sweep is in gray lines. Gain = 1.

Enable Triggering of Constant Amp Pulses

1 Phase2 Dur (x0-x4) 2000us

0 Pulse Amplitude (V) 0.1 Manual Increment (V) Polarity Tr 🛄 T

Appendix 4 Suggestions for Future StimJim Capabilities

Appendix 4.1 Link a StimJim Isolator Program to a Particular StimJim by Reading its Serial Number

This would be useful (and perhaps crucial) for unambiguously connecting one of many StimJim Isolator programs to a particular StimJim.

Appendix 4.2 Read Cermak's Internal StimJim Teensy Program Version Number

Read Cermak's Internal StimJim Arduino/Teensy Program Version Number to pair the StimJim Isolator program code with the version of the Arduino/Teensy program in the StimJim.

Appendix 4.3 Measure Electrode Current and Voltage Simultaneously

If StimJim could measure the electrode's current being passes and the voltage applied at the same time, there would be no need to go to the compliance voltage to calculate the electrode resistance. Electrode resistance could be measured for each pulse. But you still would have to go to the compliance voltage to measure the Maximum Current the electrode is capable of passing.

Appendix 4.4 Increase the compliance voltage to 27 Volts or, Even Better, 54 Volts

This is the most imoirtant possible upgrade.