FEDERAL UNIVERSITY OF SÃO JOÃO DEL-REI – UFSJ

NEUROENGINEERING AND NEUROSCIENCE INTERDISCIPLINARY LABORATORY – LINNCE

OPEN STIM ELECTRICAL TESTING MANUAL

Brazil, May 2021

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This manual will assist in testing Open Stim. They are electrical tests, to be carried out on bench with oscilloscope and multimeter. It is possible to check the frequency, the stimulation current, pulse duration, pulse phase, slew rate, among others.

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SUMMARY

CHAPTER 1

INTRODUCTION AND OBJECTIVES

The tests are of fundamental importance to verify all the functionalities and limitations of the hardware and software. As it is a prototype, we do not have any tests performed on animals yet. Thus, all the tests were carried out in an electronics laboratory, simulating the brain or tissue with electrical resistors.

These tests enable us to verify that the configured parameters are actually being delivered by the stimulator. In addition, we want to know the percentage of error contained in each parameter, to establish its acceptable margins, and to find limitations. After all the tests are done and results are consolidated and that the values found meet the need for your research, there is the possibility of tests on animals. Bear in mind that, for tests on animals, approval from the institution's ethics committee is required before starting any experimental procedure.

The main purpose of this manual is explain how to check the frequency, pulse duration, pulse phase, electrical current and slew rate being delivered by the stimulator.

CHAPTER 2

TESTING EQUIPMENT

To test Open Stim, we used the Agilent oscilloscope model DSO-X 2002A, which is shown in Figure 1. However, any oscilloscope with basic functions is capable of carrying out the necessary tests.

Figure 1 - Agilent DSO-X 2002A Oscilloscope

The multimeter used was the Agilent model U1241B, which is in Figure 2. It is important that the multimeter is capable of measuring microamperes and has good accuracy, since the stimulator supplies very low output currents. Before starting to measure the currents, check the maximum current that the multimeter can measure, and also check that the low current fuse is not open.

Another question that is a common mistake is the multimeter's probes. Check if they are connected to the multimeter for current measurement. Also confirm the current scale to be used, which is direct current in microamperes.

Finally, check the batteries of the multimeter, as it will be used for a long period of time. Remove and measure all and, if necessary, replace them.

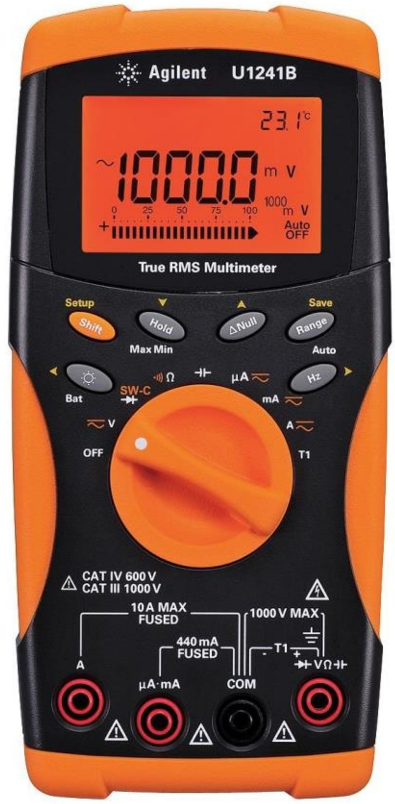

Figure 2 - Agilent U1241B Multimeter

We also recommend having on the bench some cables for jumpers, alligator claws, cutting pliers, stripper pliers, electrical tape, note paper, pen, calculator, in short, everything that will facilitate the quality of the tests.

CHAPTER 3

FREQUENCY TEST

To check the accuracy of the frequency, only the oscilloscope is needed. Consider 5 % error for the frequency, this value meets the requirements of the project.

To carry out this test, put a resistance on the output, between 1K and 10K that will simulate the brain of the animal. Place the ends of the oscilloscope in this output resistor. Now turn on the stimulator, enter frequency values between 0.1 Hz and 300 Hz by typing the value on to the Nextion screen. Of course, also entering the pulse width values is required, but at this moment, we will not be checking this parameter, only the frequency. Enter large values of pulse width, such as 50% of the duty cycle (period between firing of pulses), to facilitate viewing on the oscilloscope screen. For example: for a frequency of 20 Hz, the largest pulse width is 50 ms However, as they are biphasic pulses, we have 25 ms for each pulse. Therefore, an average value to be entered for pulse width for the frequency of 20 Hz would be 12.5 ms. Table 1 contains the frequency results measured and the margin of error found for each value.

Table 1 - Frequency

FREQUENCY (Hz)	MEASURED FREQUENCY (Hz)	ERROR (%)
300	299.19	0.27
250	249.60	0.16
200	199.63	0.18
150	149.78	0.15
100	99.95	0.05
90	89.92	0.09
80	79.85	0.19
70	69.86	0.20
60	59.84	0.26
50	49.93	0.14
40	39.99	0.02
30	29.96	0.13
20	19.94	0.30
15	14.96	0.27
10	9.99	0.1
9	8.99	0.11
8	7.99	0.12
7	6.98	0.29
6	5.99	0.17
5	4.99	0.20
4	3.99	0.25
3	2.99	0.33
2	1.99	0.50
1	0.99	1.0
0.5	0.48	4.17

If the frequencies are not within the expected levels, check the connections in order to reduce or eliminate noise. Another point is to check the Arduino code, in case the difference is very large, obtaining an error greater than 5%. Below are some screenshots of the oscilloscope, showing the values found in the Open Stim built at UFSJ, by LINNCE.

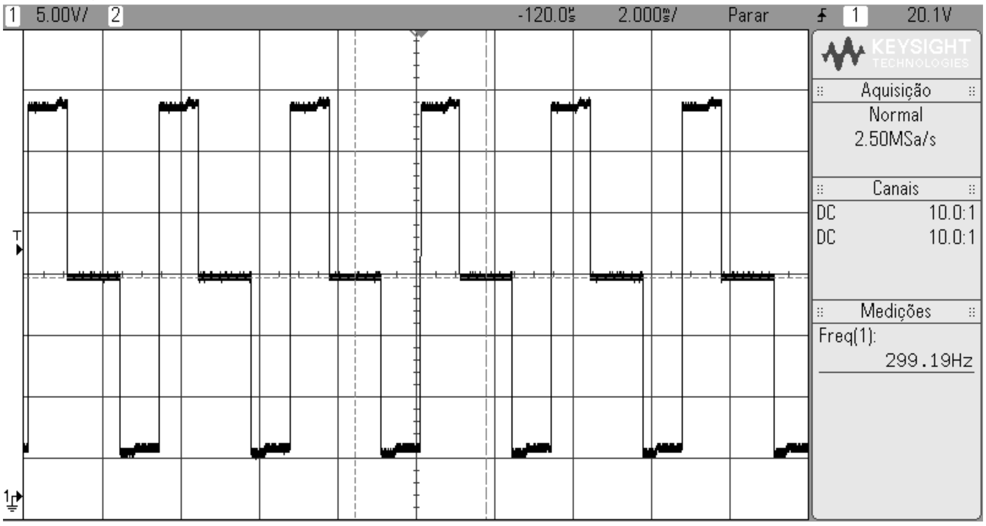

Figure 3 - Frequency of 300 Hz

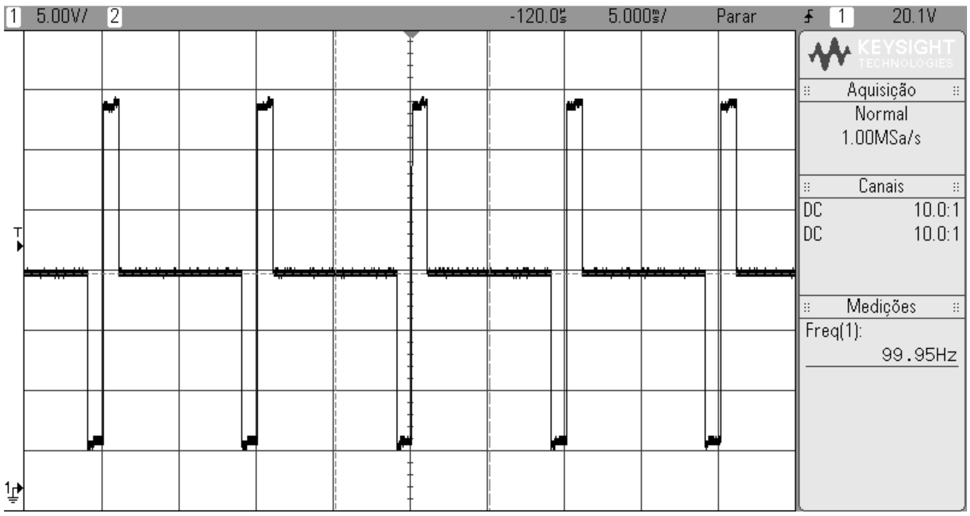

Figure 4 - Frequency of 100 Hz

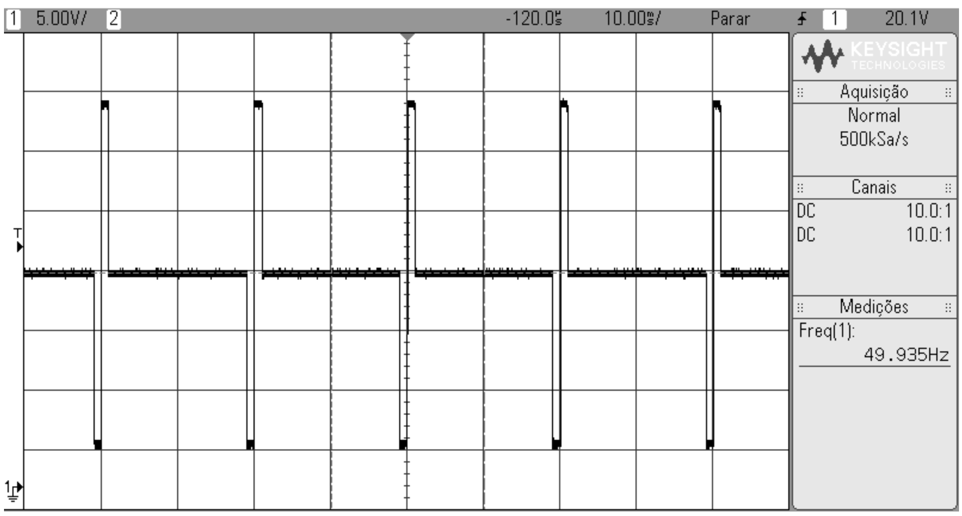

Figure 5 - Frequency of 50 Hz

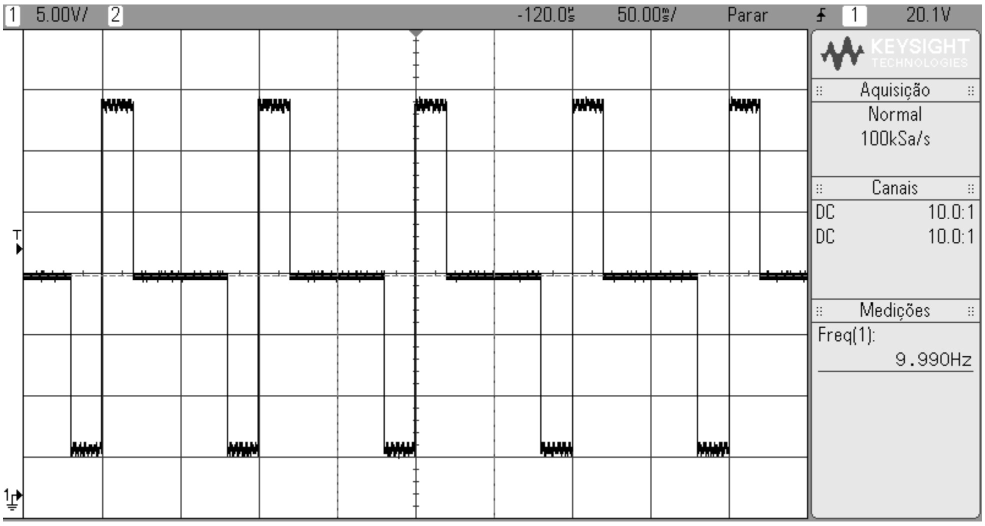

Figure 6 - Frequency of 10 Hz

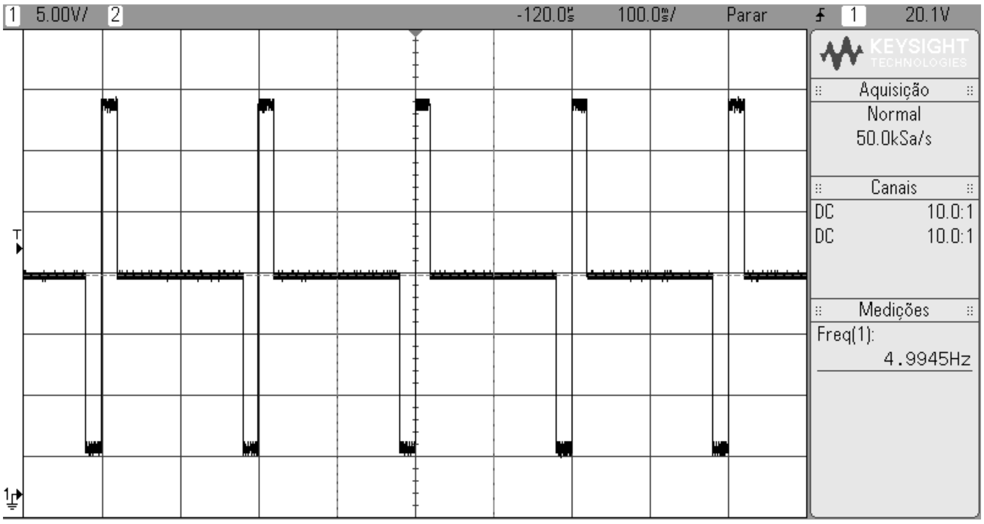

Figure 7 - Frequency of 5 Hz

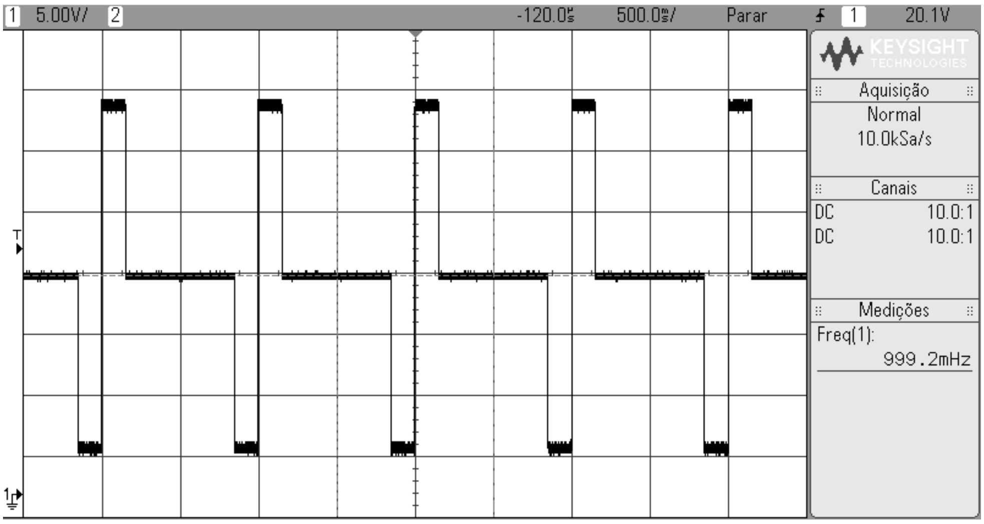

Figure 8 - Frequency of 1 Hz

CHAPTER 4

PULSE WIDTH TEST

The pulse width test follows the same reasoning as the frequency test: enter the value on the Nextion screen and check the value on the output with the oscilloscope and considering a margin of error of 5 %. Table 2 shows some results found.

Table 2 - Pulse Width

PULSE WIDTH (ms)	MEASURED POSITIVE PULSE WIDTH (ms)	ERROR (%)	MEASURED NEGATIVE PULSE WIDTH (ms)	ERROR (%)
4 µs (@280 Hz)	4.18 µs	4.31 %	5.08 µs	21.26 %
64 µs (@20Hz)	64 µs	0 %	65.2 µs	1.84 %
500 µs (@2 Hz)	514 µs	2.72 %	514 µs	2.72 %

The following screenshots show the values mentioned above. The values are found with the aid of the oscilloscope cursor tool, over the X.

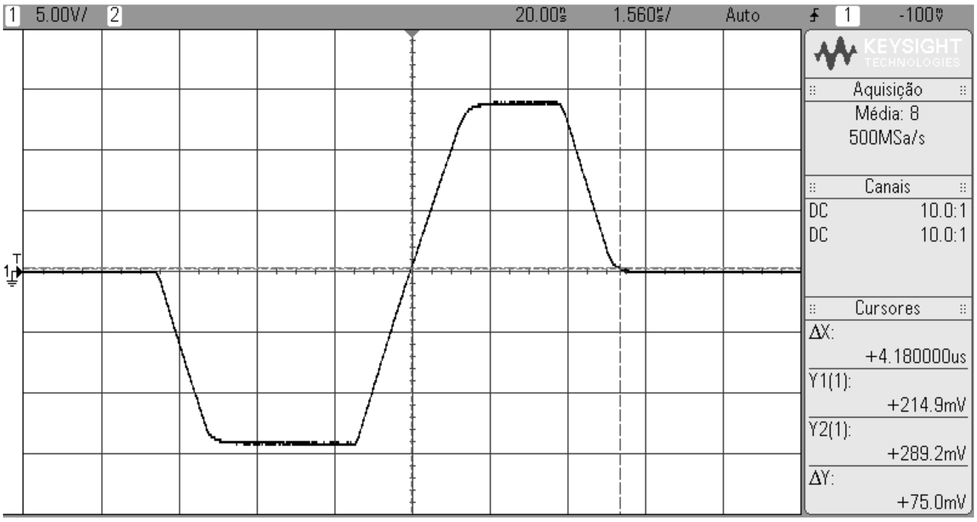

Figure 9 - Positive Pulse Width 4 µs

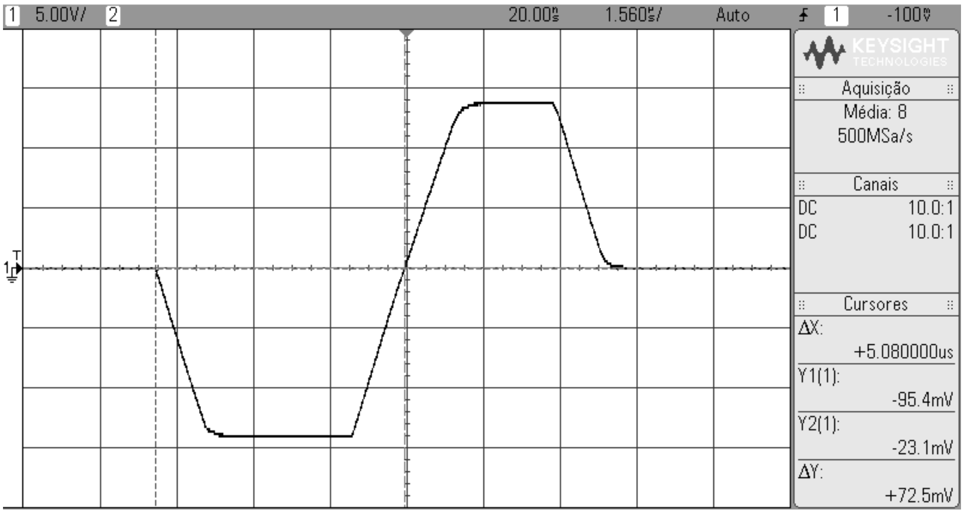

Figure 10 - Negative Pulse Width 4 µs

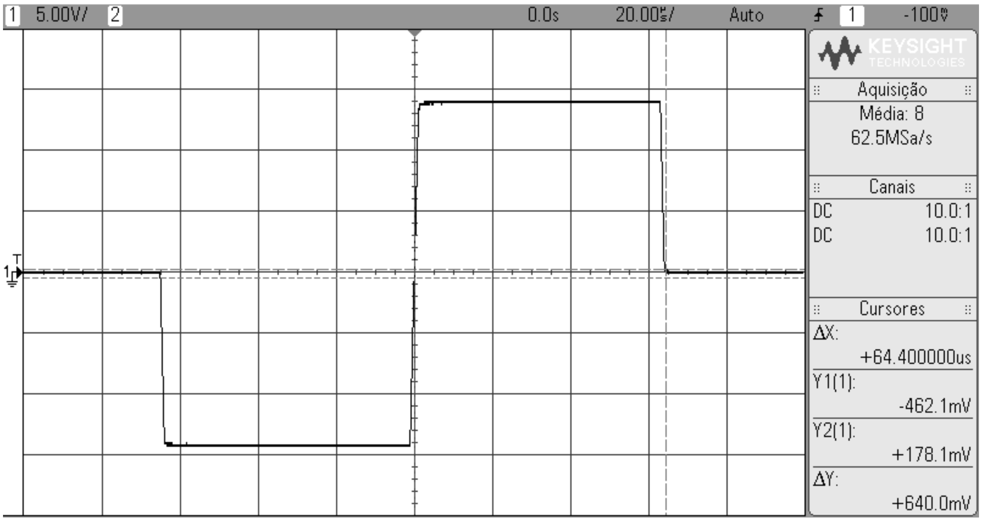

Figure 11 - Positive Pulse Width 64 µs

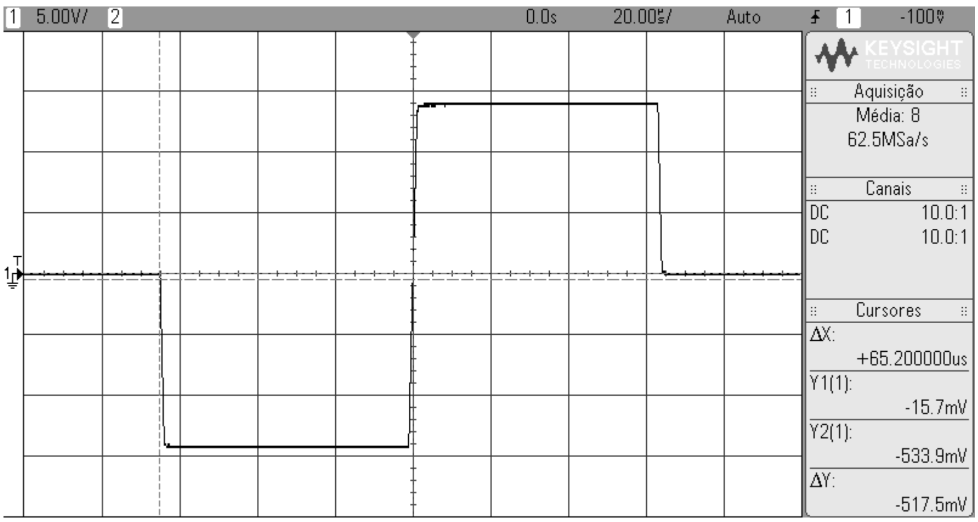

Figure 12 - Negative Pulse Width 64 µs

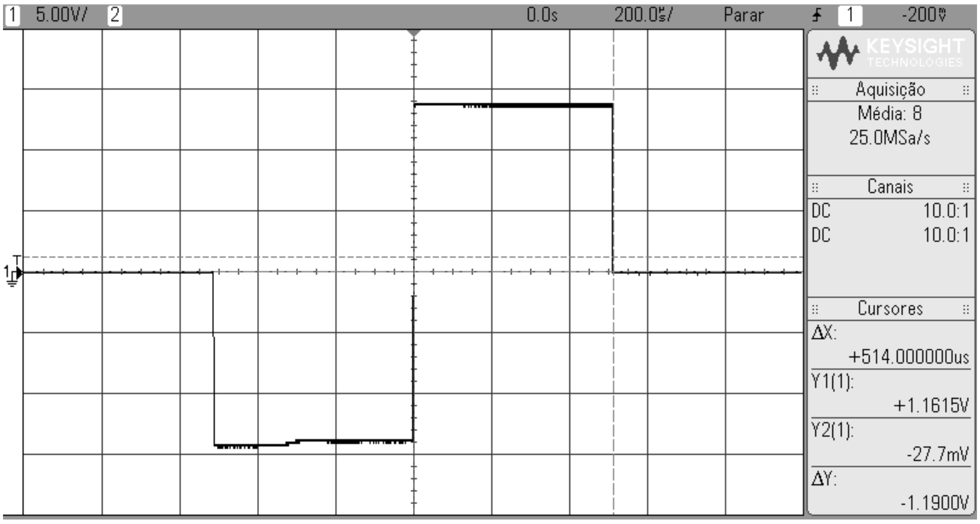

Figure 13- Positive Pulse Width 500 µs

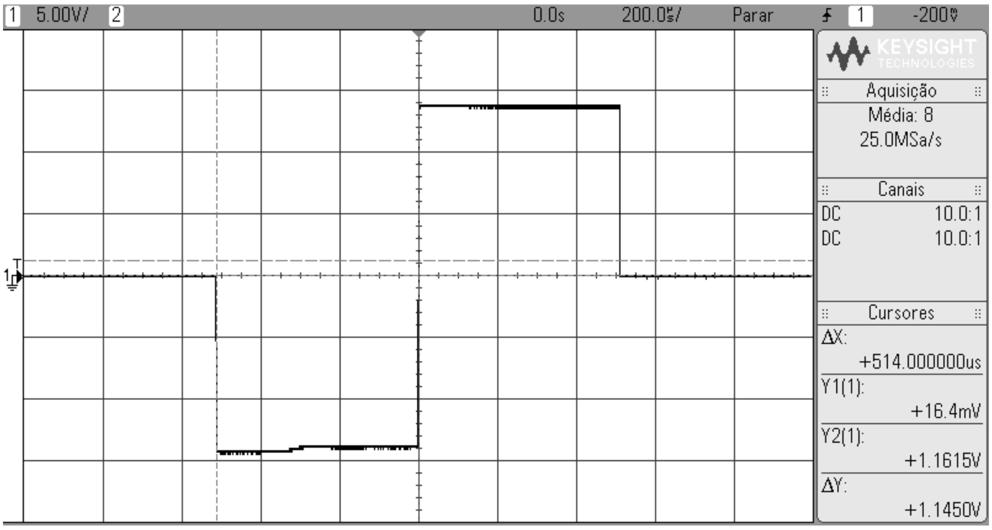

Figure 14 - Negative Pulse Width 500 µs

4.1 – PULSE WIDTH vs. FREQUENCY

As described in the Open Stim manual, the device is operated by means of PWM. Thus, it depends solely on the 16 MHz internal clock of Atmega 32u4 processor. In this sense, it is important to understand PWM operation and configuration in this processor to fully grasp the link between these two parameters (pulse width and frequency), how they interfere with each other, and what is the limitation of such form of operation.

When lower frequencies are desired, the frequency of the internal clock can be divided by factors expressed as powers of base 2 (2^N) using the prescaler function (see chapter 15 of the ATMEGA 32u4 datasheet). For instance, setting the prescaler to 1 yields the original frequency, that is, 16 MHz. When the prescaler is 2, we get 8 MHz, and so on. Now, to obtain frequency values between the original frequency and its by-2 divisions, it is necessary to count a fixed quantity of clock pulses. This is done by the internal Atmega 32u4 10-bit counter. Thus, for instance, if we use a prescaler of 64 and a count of 1024 pulse, we obtain a frequency of 244.14 Hz.

$$\frac{250x10^3}{1024} = 244.14\ Hz \tag{1}$$

Using the same prescaler, but counting 833 pulses instead, we get 300.12 Hz. Table 3 below shows, according to the prescaler chosen, the new (divided) base frequency, the minimum possible frequency (when 1024 pulses are counted), and the maximum value of frequency used in this project (which is the minimum of the next prescaler). We only show prescaler values used in our programming.

Configuration of pulse duration uses exactly the same clock system, including the prescaler and the 10-bit counter. That means widths are limited to multiples of the pulse duration of the divided clock signal. For instance, by using a prescaler value of 64, which yields a divided frequency of 250 KHz, the minimum duration of a pulse will be 1/250000 Hz = 4 ms, which is shorter than most experimental applications in neuroscience. On the other hand, if slower firing frequencies are desired, only longer pulse durations will be available. For instance, if a prescaler of 16384 is used, the base frequency becomes 976,5625 Hz. In this case, the lowest frequency is circa 0.95 Hz with the shortest pulse duration of 1.024 ms. Table 4 shows minimum pulse width obtainable for each prescaler.

In our experience, the range of values made available by this technology is sufficient for a very wide range of experimental protocols used in neuroscience and also in other areas of physiology (e.g., muscular and cardiac). If needed, one should consider developing of a derivative of Open Stim using Arduino boards with better performance. For instance, Arduino Due has an internal counter of 32 bits which will greatly improve the granularity of pulse widths and frequency ranges. It is important to highlight, though, that Arduino Due is not readily interchangeable with Leonardo, given, among other technical aspects, differences in operation voltages (3.3 V vs. 5 V).

Table 3 - Frequency limits

PRESCALER	NEW FREQUENCY (Hz)	MINIMUM FREQUENCY (Hz)	MAXIMUM FREQUENCY (Hz)
64	250000	244.14	488.28
128	125000	122.07	244.14
256	62500	61.03	122.07
512	31250	30.52	61.03
1024	15625	15.26	30.52
2048	7812.5	7.63	15.26
4096	3906.25	3.81	7.63
8192	1953.125	1.91	3.81
16384	976.5625	0.95	1.91

Table 4 - Limit of pulse width by frequency range

PRESCALER	MINIMUM FREQUENCY (Hz)	MAXIMUM FREQUENCY (Hz)	MINIMUM PULSE WIDTH (ms)
64	244.14	488.28	0.004
128	122.07	244.14	0.008
256	61.03	122.07	0.016
512	30.52	61.03	0.032
1024	15.26	30.52	0.064
2048	7.63	15.26	0.128
4096	3.81	7.63	0.256
8192	1.91	3.81	0.512
16384	0.95	1.91	1.024

For frequencies below 0.95 Hz, it is necessary to use the Arduino delay function and it is no longer possible to do it directly using PWM. Using the delay function, the processor halts the execution of the program until the commanded time has passed, which causes system unresponsiveness. That is why such approach was avoided and used only when absolutely necessary (frequencies below 0.95 Hz). Users and builders should bear this in mind when using Open Stim or creating its derivatives.

CHAPTER 5

CONSTANT CURRENT TEST

The constant current test consists in verifying that output current stays the same even if the output impedance changes, for instance, when performing stimulation to different animals. Although simplistically, we can approximate the equivalent resistance of Wistar rats' brains to a range between 1 kΩ and 10 KΩ on average. Thus, we performed the tests considering values between 1 KΩ and 10 KΩ, which were represented by a resistor connected to the stimulator output.

With the multimeter connected in series with the output resistance and on the direct current scale, proceed as follows:

1) Define the first value of output resistor with the first possible value being 1 KΩ and the last possible value being 10 KΩ.

2) Define the resistor of the resistor board, that is, R10, the first possible value being 4.02 KΩ and the last 360 KΩ.

3) We suggest that you start as follows: Set the output resistance to 10 KΩ, which is the maximum value, and vary R10 from 4.02 KΩ to 360 KΩ using the rotary switches. In this way, all currents for output equal to 10 KΩ are measured. Note the positive and negative current values. In order to obtain the correct current and not the average RMS, set a low frequency with a large pulse width. In this way, the multimeter is able to make the correct measurement. If you prefer, it is also possible to do it by assessing the voltage in the output resistor and using Ohm's law relation to obtain the current.

4) After taking the measurements for R11 equal to 10 KΩ, change the resistance to a lower one, in this work we use the following values: 1 KΩ, 2 KΩ, 4.02 KΩ, 6.12 KΩ, 7.15 KΩ, 8.25 KΩ and 10 KΩ. Follow in the same way as mentioned above and measure the positive and negative currents to the value of 8.25 KΩ, for example. Then to 7.15 KΩ, 6.12 KΩ, 4.02 KΩ, 2 KΩ, 1 KΩ.

5) Now with all measured currents, calculate the error between the maximum and minimum positive current and then the error between the maximum and minimum negative current. This error cannot exceed 10%.

Table 5 and Table 6 show the positive and negative currents, respectively, with the values found by the authors of the project, as well as their error.

Table 5 - Positive current measurement

R10 (KΩ)	1 KΩ	2 KΩ	4,02 KΩ	6,19 KΩ	7,15 KΩ	8,25 KΩ	10 KΩ	ERROR
4,02	1252	1250	1247	1245	1244	1240	1239	1,04%
4,7	1068	1065	1064	1062	1061	1058	1050	1,69%
5,6	902	900	896	894	892	890	885	1,88%
6,19	810	806	805	804	803	800	795	1,85%
7,15	702	700	697	695	694	692	686	2,28%
8,25	609	606	605	604	604	600	595	2,30%
10	505	502	500	500	498	495	491	2,77%
12,1	415	414	413	413	411	405	400	3,61%
14	360	357	355	355	354	350	347	3,61%
16,9	302	300	298	295	294	291	288	4,64%
20,5	250	248	245	243	243	241	240	4,00%
24,9	208	206	205	202	199	197	195	6,25%
33,2	154	153	153	152	150	149	148	3,90%
44,2	115	114	112	110	111	110	108	6,09%
56,2	92	91	90	90	88	88	86	6,52%
61,9	83	83	82	81	80	79	77	7,23%
71,5	72	71	70	70	69	68	67	6,94%
78,7	64	64	63	63	62	62	61	4,69%
100	51	51	50	50	49	47	47	7,84%
120	43	42	42	41	41	40	39	9,30%
150	34	34	33	33	33	33	32	5,88%
240	22	22	22	21	21	21	20	9,09%
360	15	15	15	14	14	14	14	6,67%

Table 6 - Negative current measurement

R10 (KΩ)	1 KΩ	2 KΩ	4,02 KΩ	6,19 KΩ	7,15 KΩ	8,25 KΩ	10 KΩ	ERROR
4,02	1245	1241	1239	1238	1238	1238	1235	0,80%
4,7	1059	1059	1057	1055	1055	1054	1048	1,04%
5,6	897	895	892	890	890	888	885	1,34%
6,19	807	805	803	802	800	799	795	1,49%
7,15	695	694	692	691	690	687	685	1,44%
8,25	605	604	603	602	601	599	596	1,49%
10	500	498	496	495	495	493	490	2,00%
12,1	413	411	410	408	408	405	400	3,15%
14	357	355	354	353	352	350	345	3,36%
16,9	300	297	295	293	292	291	290	3,33%
20,5	247	245	244	242	242	240	238	3,64%
24,9	205	202	200	199	198	197	195	4,88%
33,2	152	150	149	149	149	147	145	4,61%
44,2	115	114	112	112	111	110	108	6,09%
56,2	91	91	90	88	87	87	85	6,59%
61,9	82	81	80	80	79	79	77	6,10%
71,5	72	71	70	70	69	68	67	6,94%
78,7	64	64	63	62	62	61	60	6,25%
100	51	50	50	49	49	48	48	5,88%
120	42	42	41	41	40	39	39	7,14%
150	34	34	34	33	33	32	31	8,82%
240	22	22	21	21	20	20	20	9,09%
360	14	14	14	13	13	13	13	7,14%

CHAPTER 6

SLEW RATE

The Slew Rate is the rate of change of the output voltage over time, according to equation 6, with its unit of measurement V / µs.

$$SR = \frac{dV_0}{dt} \qquad (2)$$

The slew rate of Open Stim is limited to the properties of the operational amplifier, which cannot change its output voltage instantly when there is a change in the input voltage, as shown in Figure 15. Therefore, if the input signal is to demand a response at the output with a rate greater than the value specified in the slew rate, the amplifier will not respond as expected.

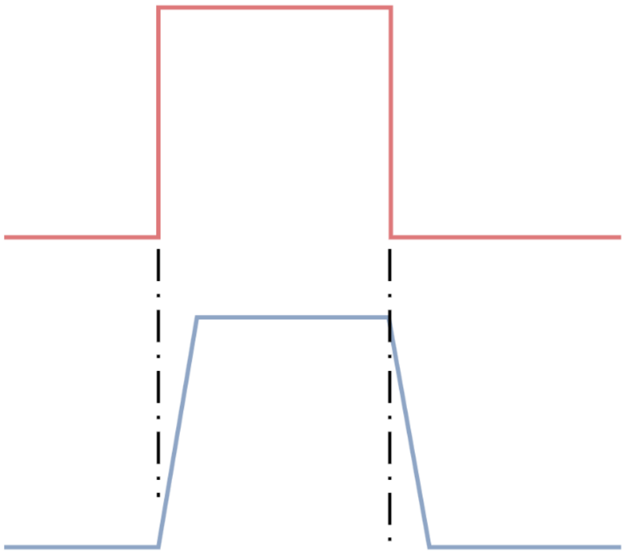

Figure 15 - Slew Rate: Response of the output signal in relation to the input

Ideally, to measure the slew rate, we take the interval between 10% and 90% of the signal change curve. That is, we disregard the first 10% and the last 10% to avoid noise fluctuations and curving of baseline that could impair reading. Figure 16 shows how it is done.

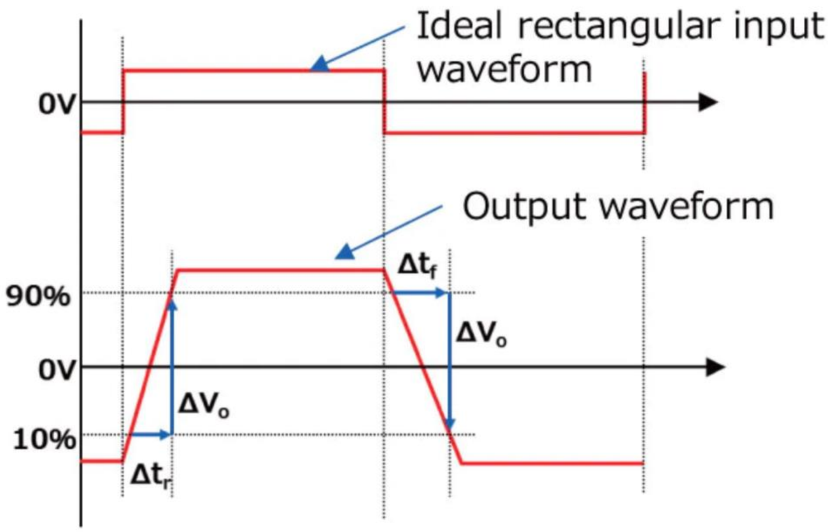

Figure 16 - Slew Rate: measurement between 10% and 90% of the characteristic curve to obtain Δt e ΔV_0

Following are the prints of the slew rate measured at the Open Stim output. The stimulator was set to 50 Hz and 8 ms to perform all tests. The operational amplifier TL 072 has a slew rate of 13 V/µs in 25˚ C, according to the datasheet. Figure 17 shows the slew rate for a positive pulse at the rising edge and Figure 18 for the positive pulse at the falling edge. Logically, for this, the negative pulse was deactivated, where the lowest possible voltage is zero volts.

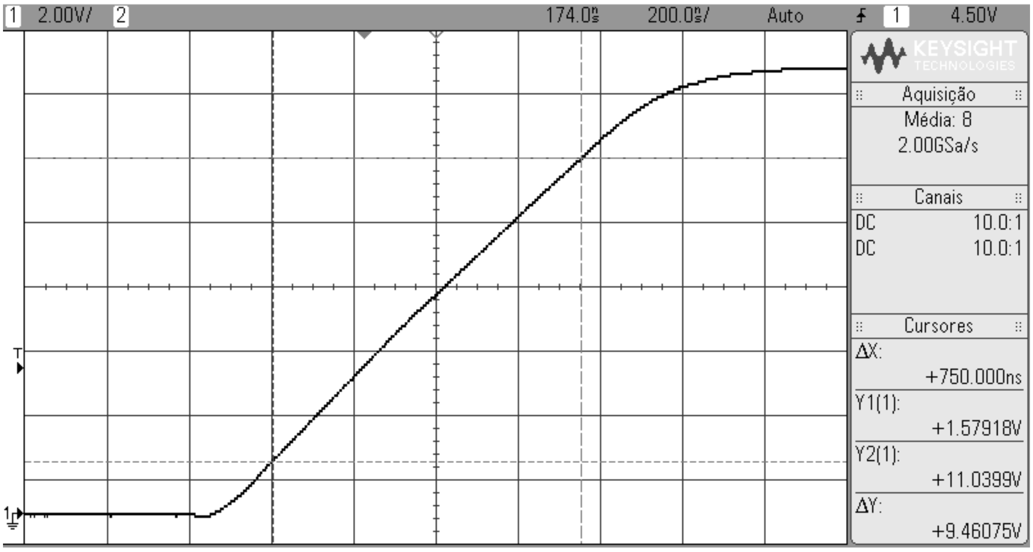

Figure 17 – $\Delta V_0 = 9.46$ V and $\Delta t = 750$ ns

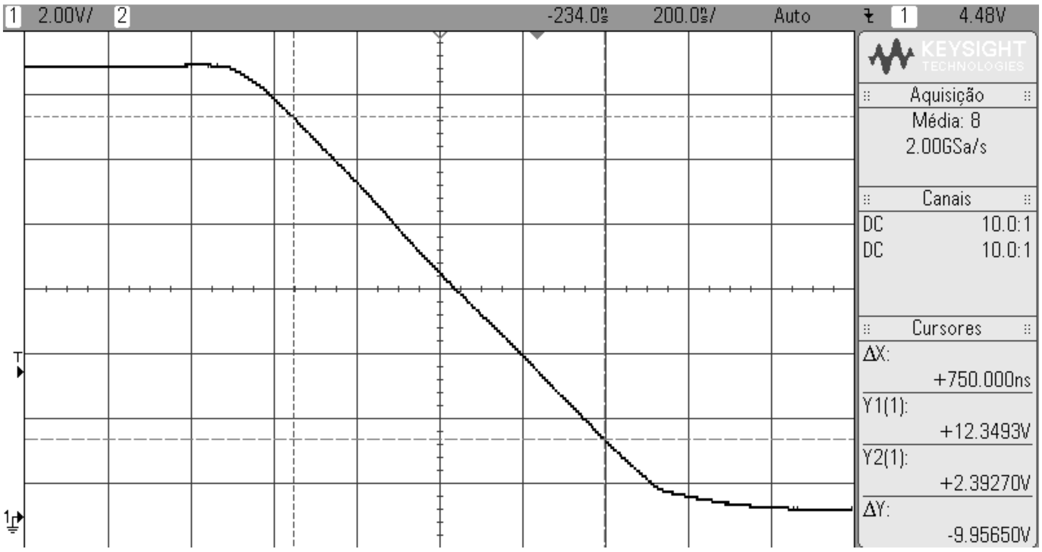

Figure 18 – $\Delta V_0 = 9.96$ V and $\Delta t = 750$ ns

In Figure 19, we keep the frequency and pulse width settings, disable the positive pulse, and enable the negative pulse. In this case, we have a rising edge with a negative pulse. In Figure 20, the setup is similar but the falling edge is assessed.

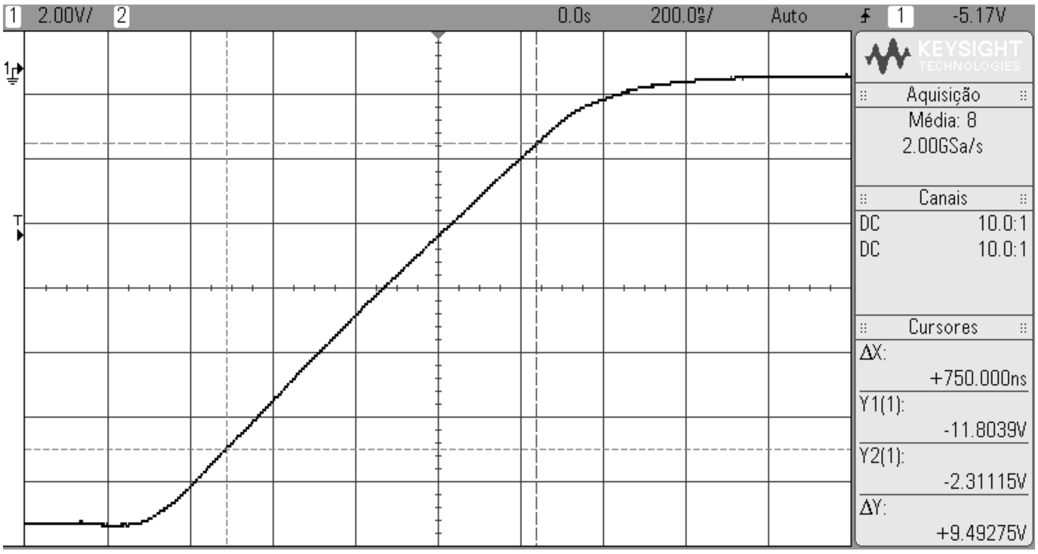

Figure 19 – $\Delta V_0 = 9.49$ V and $\Delta t = 750$ ns

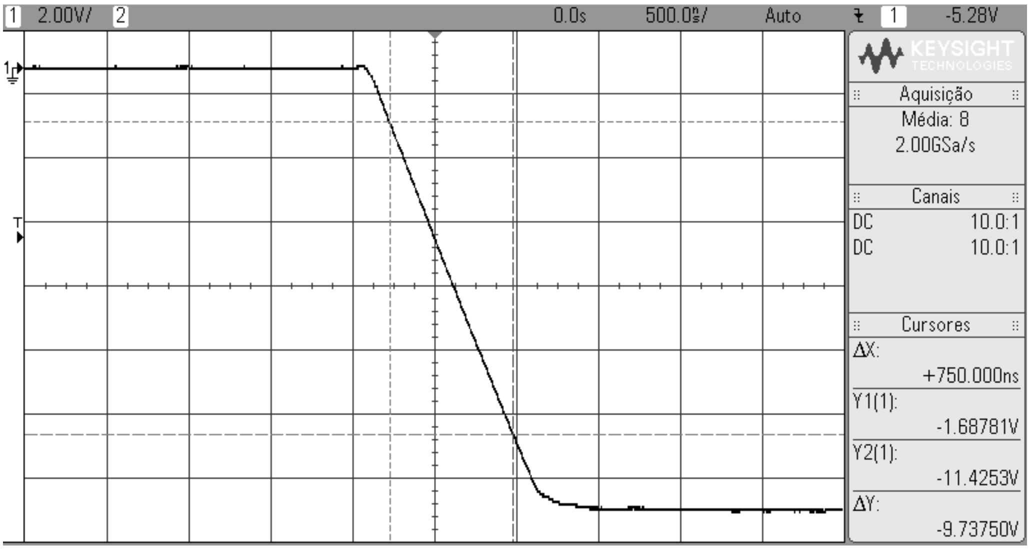

Figure 20 – $\Delta V_0 = 9.74$ V and $\Delta t = 750$ ns

In Figure 21, we have both pulses enabled, that is, biphasic pulses, with rising edge.

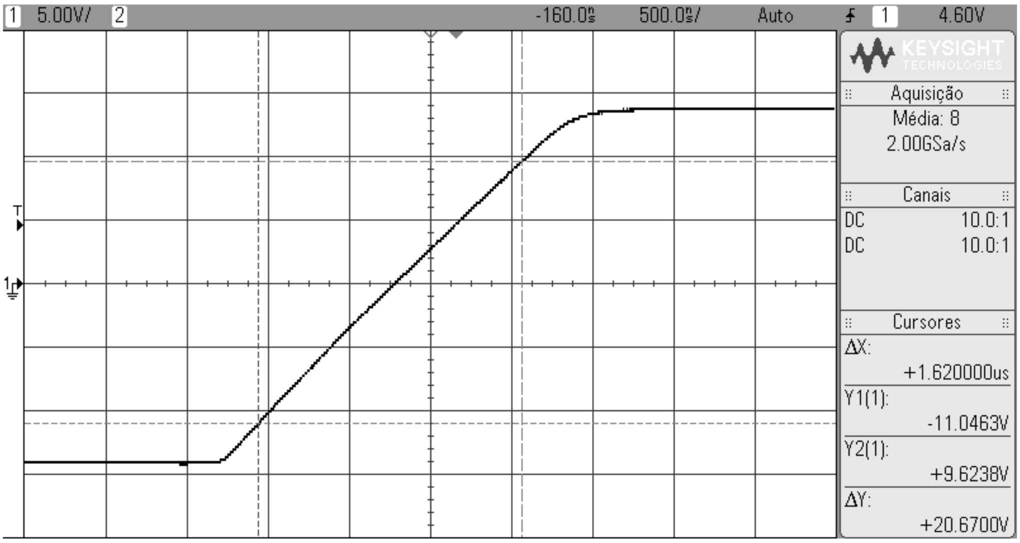

Figure 21 – $\Delta V_0 = 20.67$ V and $\Delta t = 1.62$ µs

For each Figure, we calculate the slew rate found, remembering that the unit of measurement is V/µs.

To Figure 17:

$$SR = \frac{9.46}{750000} = 12.61\, V/\mu s \tag{3}$$

To Figure 18:

$$SR = \frac{9.96}{750000} = 13.28\, V/\mu s \tag{4}$$

To Figure 19:

$$SR = \frac{9.49}{750000} = 12.65\, V/\mu s \tag{5}$$

To Figure 20:

$$SR = \frac{9.74}{750000} = 12.99\, V/\mu s \tag{6}$$

To Figure 21:

$$SR = \frac{20.67}{1.62} = 12.76\, V/\mu s \tag{7}$$

Values obtained are, as expected, very close to those described in the operational amplifier (TL072) datasheet. It noteworthy that the chosen IC, in addition to having low noise, presents a great slew rate, in face of its low cost (e.g., nominal slew rate of LM741 is only 0.5 V/ s slew rate, although costs are similar). If faster output are needed, one should consider interchanging the IC for superior models, while considering the impact on costs.

CHAPTER 7

DEAD TIME

As we have already seen in the case of the slew rate, we also have dead time, this delay essentially depends on the distance between the points and the speed with which the transfer is made. The shorter the distance between the points, the shorter the delay time, the higher the transfer speed, the shorter the dead time. In other words, the dead time, is the time interval between the instant when the system undergoes any variation and the instant when it begins to respond to that variation.

To start analyzing the dead time on the Open Stim circuit, we look at Figure 22, which presents an example of dead time on any system.

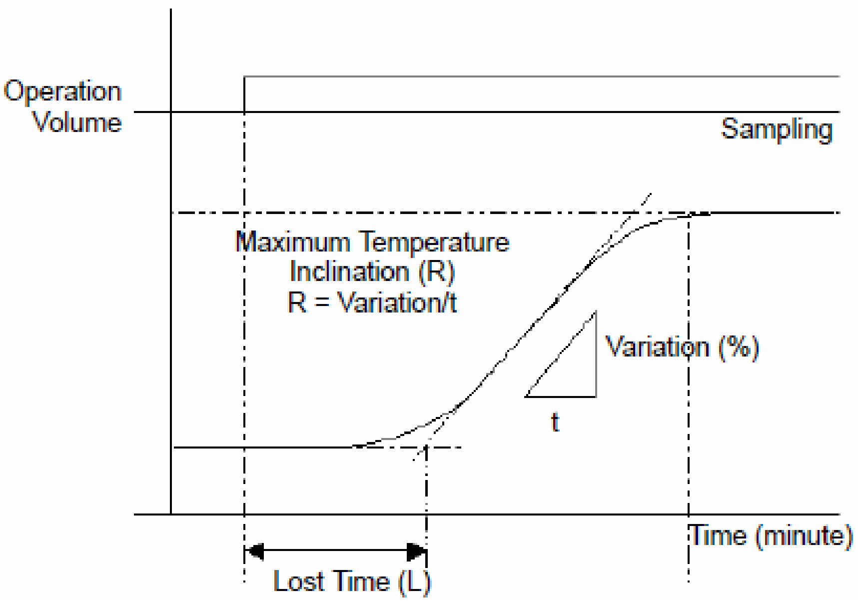

Figure 22 - Example of dead time

In Figure 23, we have a positive and negative pulse (anodic and cathodic), however, without the necessary zoom to see in detail the slew rate or dead time. In Figure 24 we have a zoom of Figure 23, where it is already possible to notice that there is a slew rate, as shown in the previous chapter, but we still don't notice dead time.

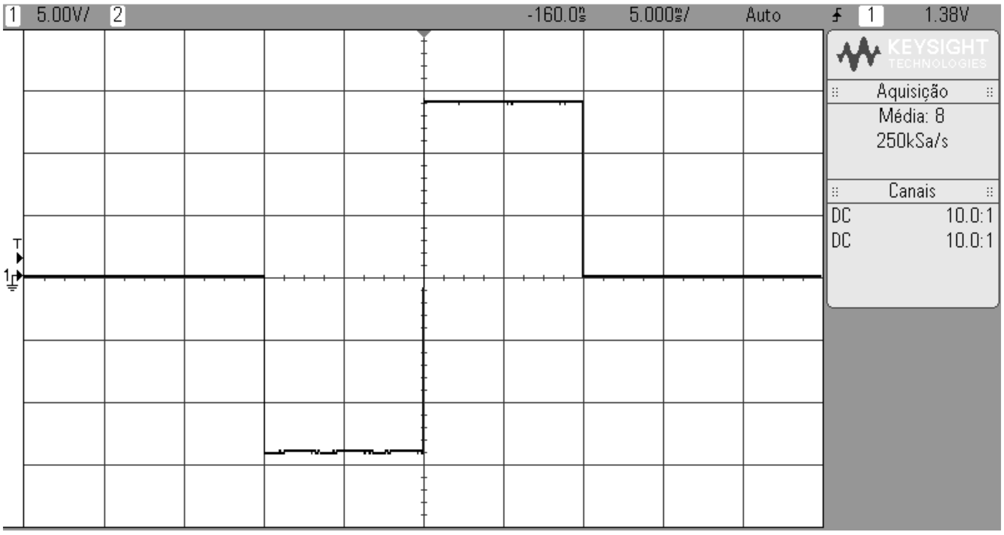

Figure 23 - Positive and negative pulse (anodic and cathodic)

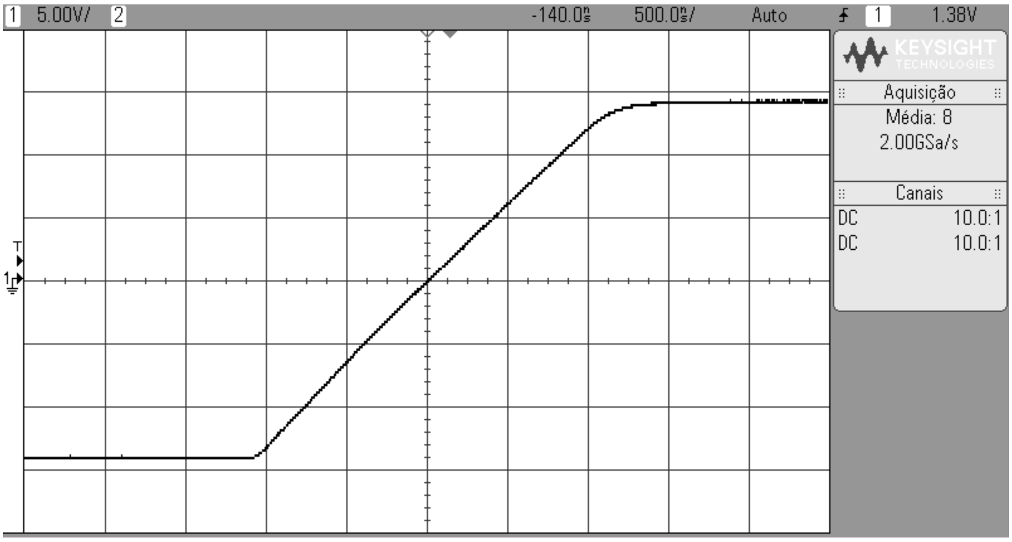

Figure 24 - Positive and negative pulse (anodic and cathodic) – Zoom of the positive pulse

In Figure 25, the zoom has been enlarged even further, and here it is already possible to say that the dead time of the circuit is very close to zero. This is probably due to the choice of using Arduino’s PWM function to generate the pulses.

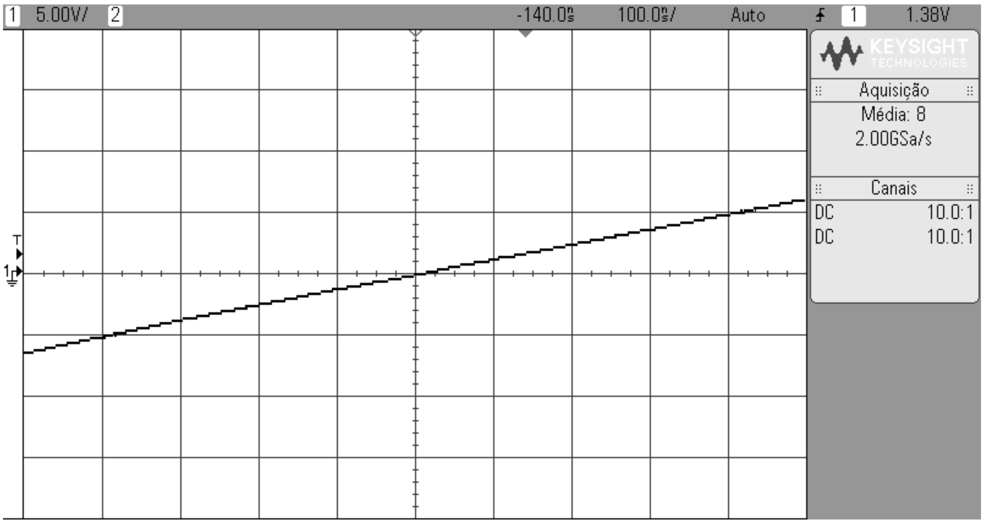

Figure 25 - Magnified zoom of the positive pulse: dead time is zero

FINAL CONSIDERATIONS

The Laboratory of Neuroengineering and Neuroscience - LINNce of the Federal University of São João del-Rei - UFSJ, hopes that, with this equipment and with all the tests carried out, you can start or continue your research inside or outside a university or technical school.

If, during the tests, the stimulator shows any non-standard results, first check the cables, solders and batteries, and then change the components. If the problem is still not resolved, return to the assembly manual. If you encounter any limitations or technical mistakes in our design, testing, and application, please let us know by discussing it in the repository forum. This will help us develop a more robust and safer tool for electrophysiological experiments.

We appreciate your interest in getting to know our stimulator and we count on your contributions to improve the project through GitHub. See you soon and good studies!

ACKNOWLEDGMENTS

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