<u>The Role of Voltage Gated Potassium Channels in *Hirudo medicinalis* Segmental <u>Ganglion Pressure Neurons</u></u>

> Allison Goodwin-Wilson 260357581 Partner: Daryan Chitsaz

#### Abstract:

4-aminopyridine (4-AP) is a pharmacological blocker of voltage gated potassium channels involved in repolarization of the cell membrane after an action potential. When voltage gated potassium channels have been blocked, neurons generate larger, longer action potentials. Because of its role in neural excitation, 4-AP has been used as a model for epilepsy and as a treatment for multiple sclerosis. In the following experiments, the effect of 4-AP on leech ganglion pressure (P) cells is investigated. We found that 4-AP increases the amplitude and duration of action potentials at different current levels. Surprisingly, it also caused a decrease in input resistance. 4-AP showed a slight increase in the time constant,  $\tau$ , suggesting that it may have effects on passive membrane properties as well.

## Introduction:

*Hirudo medicinalis* has been widely used in studies examining neuronal and neuroethological characteristics such as connectivity, membrane properties, and the behaviour associated with them (Kristan et al., 2005, Ready et al., 1979). The leech is a good candidate for such experiments because of the relative simplicity, accessibility (Kristan et al., 2005), and stereotypical nature of its neurons and circuitry (Fig. 1), as well as the behaviour associated with them. This behaviour is often rhythmic or oscillatory (Fuchs et al., 1980, Kristan et al., 2005). In order to understand the neural circuits and related behaviour of higher animals and humans, it is often useful to have a less complicated model to study. The medicinal leech provides researchers with such a model and continues to allow for the simplified study of cellular neural properties.

Several cell types have been identified and are easily found over and over again in the leech segmental ganglia. These include pressure (P) cells, nociceptive (N) cells, touch (T) cells, and the two large, central Retzius cells (Fig. 1) (Fuchs et al., 1980). Each type of cell has its own, unambiguous profile of electrical properties. The three sensory



**Fig. 1: Leech anatomy** A) shows the organization of the leech, including its twenty-one segmental ganglia. B) shows one of these ganglia, with cell types labeled: purple - P cell, blue - R cell, green - T cell, red - N cell

cells have large action potentials. P cells are the largest of the sensory cells and fire action potentials when stimulated during depolarization or after hyperpolarization (Nicholls et al., 1968, Gerard et al., 2012). N cells fire single action potentials with large undershoots (Nicholls et al., 1968). T cells fire bursts of action potentials during maintained depolarization and are smaller than N or P cells (Nicholls et al., 1968). Retzius cells are involved in the relaxation of body wall muscles (Mason et al., 1978). These cells fire multiple, smaller action potentials when stimulated with a depolarizing current (Nicholls et al., 1968).

Many channels play a role in generating action potentials by changing the cell membrane's permeability at different times. Voltage gated potassium (Kv) channels (also called delayed rectifier potassium channels) are one of these (Fig. 2), and are involved in regulating the resting potential, repolarization, and the frequency at which cells fire (Chi et al., 2007). The drug 4-aminopyridine (4-AP) (Fig. 2) blocks open Kv



channels to ease the generation of and prolong action potentials in neurons (Boiko et al., 2013, Schechter, 1997, Judge et al., 2006). In the following experiment we characterized the action of 4AP on Kv channels in different leech ganglion cells,

Fig. 2: A) The chemical structure of 4-Aminopyridine and B) the protein structre of the four-subunit voltage gated potassium channel that 4-AP blocks.

primarily P cells, which we chose because of their ability to fire multiple times with one depolarization and their size, which made them easier to access. We expected to see an increase in action potentials with the addition of the drug.

Understanding the effect of Kv channel blockers in different types of cells is a clinically relevant pursuit. Because of 4-AP's known effect of increasing neural excitability, it has also been used in models for epilepsy (Yamaguchi et al., 1992). Additionally, 4-AP's relevance to multiple sclerosis has been investigated, due to its ability to prolong action potentials (Judge et al., 2006). Studies using Kv blockers will help researchers and clinicians gain insight into these diseases.

### Methods:

We took intracellular recordings from different cells in *Hirudo medicinalis* ganglia. Prior to recording from each ganglion, we made a sketch of the cells in order to identify the same cells for recording after the addition of 4-AP (Fig. 3). While we primarily recorded from P cells, one T cell was used as well, although the data was not



presented, because of a low N (see Appendix for raw T cell data). Before penetrating a

**Fig. 3:** Sketches of two leech ganglia used in these experiments. A) shows the placement of P1, P2, and P3, while B) shows the placement of P4, P5, and T1 (whose data was not used)

cell, we checked that the electrode resistance was between 18 and 30 megaohms and zeroed the voltage reading to ease analysis. Once each cell had been identified and punctured by the electrode, we waited until any spontaneous spiking had stopped, because most healthy ganglion cells do not fire tonically. We proceeded to record the firing of the cell after either depolarization, or hyperpolarization. We used currents of -2nA, -1nA, 1nA, 2nA, and 3nA. Recordings made from these cells lasted about 3 minutes per current level and contained 35 sweeps. Following the control recordings, we identified the previously recorded cells and added 10mL of 4-AP to the dish. Using the same currents as before, we recorded the results of depolarization and hyperpolarization for 10 minutes each. Electrodes were wired to a stimulator and an amplifier, which connected to a computer. Recordings were made on Axoscope9 software from Molecular Devices. When analyzing sweeps, we used only the first action potential fired in a depolarization because it was the most consistent.

### **Results:**

Using the known properties of leech ganglion cells, we identified several P cells in each ganglion. All identified cells fired multiple action potentials when stimulated



Fig. 4: Hyperpolarization induced action potential in a P cell. -2nA current injected into cell.

with depolarizing current, and also fired an action potential after hyperpolarization; one of the hallmarks of P cells (Fig. 4) (Gerard et al., 2012). Bonferroni corrections were used for all analyses with multiple comparisons. We found that for each depolarizing current level, there was, on average, a significantly longer action potential in the 4-AP condition compared to control (p<0.05, n = 175 sweeps) (1nA control = 6.85ms vs. 4-AP = 7.61ms; 2nA control = 6.56ms vs. 4-AP = 7.17ms; 3nA control =

6.40ms vs. 4-AP = 7.28ms) (Fig. 5). We also found that within the control condition, 3nA gave, on average, the shortest duration action potential, although all three differed

significantly (p<0.05). Within the 4-AP condition, while 1nA still gave the longest action potential, 2nA gave the shortest duration rather than 3nA, although all were still significantly different from each other (p<0.05) (see Appendix for graphs and data).



**Fig. 5:** The average duration of action potentials for several current levels compared between control and 4-AP conditions. 4-AP gives rise to longer action potentials at all currents.



**Fig. 6: The average amplitude of action potentials** using several different currents and compared between control and 4-AP conditions. 4-AP increases the amplitude at all current levels

4-AP was shown to significantly increase the amplitude of action potentials for each current level (p<0.05) (1nA control = 51.72mV vs. 4-AP = 56.05mV; 2nA control = 51.08mV vs. 4-AP = 54.65mV; 3nA control = 46.52mV vs. 4-AP = 53.60mV) (Fig. 6).

Within both conditions, there was a positive relationship between current level and action potential amplitude and significant differences (p<0.05) between all currents except 1nA and 2nA in the control condition, which only showed a trending difference (p=0.06) (see

Appendix for graphs and data). Both these results, and those regarding the duration of action potentials suggest that 4-AP eases the generation of action potentials in leech P cells.

For three of the recorded P cells, we calculated the time constant ( $\tau$ ) in control and 4-AP conditions, and then determined the average  $\tau$  for each condition. To find  $\tau$ , we used one sweep at a hyperpolarizing current in each condition for each cell. We found the change in voltage as a function of time, and



**Fig. 7: Finding the time constant** using logarithmic data to obtain a straight line. Tau can be extrapolated from these graphs by drawing a horizontal line at 63% of the total rise and finding the time at which it intersects with the trendline. A) control, B) 4-AP



Fig. 8: Calculation of tau. Vertical red lines show  $\Delta V$  measurements at certain time points. Horizontal red line shows maximum  $\Delta V$ .

converted this change into  $\ln(\Delta V/e)$  in order to obtain a linear graph. We plotted  $\ln(\Delta V/e)$ against time and fitted a trendline to this data. By calculating 37% of the total rise of the trendline and subtracting it off the maximum value, we were able to estimate the time at which 63% of the neuron's electrical signal still remains, unattenuated (Fig. 7). Although no

significant difference between the two was found (p>0.05), the 4-AP condition did tend to give higher time constants than control (Fig. 7). The tendency to increase  $\tau$  suggests that when Kv channels are blocked, passive electrical signals may propagate farther along the axon before they attenuate.

Finally, we plotted an average I-V curve for the control and the 4-AP conditions by finding the voltage associated with different hyperpolarizing currents (Fig. 8). The slope of these graphs is the input resistance associated with each condition (Fig. 9). For these analyses P4 and P5 did not generate any useful data with hyperpolarizing currents, and were therefore not included. The average input resistance in the 4-AP condition (R = 2.0638MΩ) was found to significantly lower than that in the control condition (R = 17.415MΩ) (p<0.05). Surprisingly, these results suggest that in the presence of 4-AP, more current is needed to elicit a change in membrane voltage, meaning that the cell is less excitable.

### **Discussion:**

After adding the Kv channel blocker 4-AP to P cells in leech ganglia, we found that inactivation of the delayed rectifier potassium channel has several effects on action

potential generation, excitability, and perhaps even the passive properties of neurons. Understanding these properties will teach scientists about the dynamics of action potentials, but also are clinically relevant. Certain neurological disorders are marked by the overexcitability of neurons, or the failure to fire action potentials. Additionally, Kv channels are seen elsewhere in the body and have even been implicated in cardiac arrhythmias (London et al., 1998, Yang et al., 1997). Because of its widespread expression and the implications associated with its blockage, voltage gated potassium channels are interesting targets for research.



**Fig. 9: Average I-V plots** were used to determine input resistance (R). R is the slope of an I-V plot. A) control, B) 4-AP

Wheeler et al. (1996) demonstrated the effects of 4-AP on action potentials in the rat hippocampus. They showed a distinct and consistent broadening of action potentials when Kv channels had been blocked by the drug. These results have been replicated in several experiments, often involving cardiac tissue. Clark et al. reported a similar lengthening in the action potential after application of 4-AP (1996). Our results hold true to these previous findings, showing a significant increase in action potential duration once 4-AP had been washed over the leech ganglion. This effect of the voltage gated potassium channel blocker is not surprising. Kv channels are primarily responsible for

repolarizing the membrane once the voltage gated sodium channels have opened and caused the depolarization associated with action potentials. If potassium is unable to flow out of the cell, making the inside more negative, the sodium depolarization will not be counteracted, and will last longer.

Another significant effect of 4-AP that we found was an increase in action potential amplitude. Again, this effect is not unique to our experiments, and has previously been demonstrated on several occasions. Stuart and Häusser showed that in cortical pyramidal neurons, baths of 4-AP increased the amplitude of dendritic action potentials (2001). Further support of these results comes from Hoffman et al., who, while studying the K<sup>+</sup> mediated signal regulation in the hippocampus, reported that 4-AP caused a dramatic increase in action potential amplitude (1997). This increase is logical, because while the voltage gated sodium channels are still open and bringing the membrane closer to sodium's equilibrium potential, Kv channels activate and begin counteracting the depolarization. If the Kv channels could not pass potassium out, the membrane would be free to depolarize all the way to  $E_{Na}$ , around +50mV. Hindering these large action potentials is important, because more depolarization causes more neurotransmitter release, which could lead to excitotoxicity (Sattler et al., 2000).

Our data was not always significant, but it was interesting to note that the time constant,  $\tau$ , showed a tendency to increase in a bath of 4-AP. Castle et al. showed that upon application of 4-AP, cells in *Xenopus* oocytes had a 2-fold increase in  $\tau$  (1994). The time constant is a parameter that measures how quickly potential will rise or fall in a neuron, and it depends on both the resistance, and capacitance of the neuron's membrane. While our results only showed a very small difference between 4-AP and control group time constants, it would be interesting to repeat the experiment using a larger number of cells or sweeps in the calculation of the average time constants. Additionally, more accurate measurements should be taken. While our calculation of  $\tau$  was fairly precise, it required an estimation of the intersection between  $\ln(\Delta V/e)$  and 63% of the maximum  $\Delta V$ . Because of this estimation, errors were possible in our calculated  $\tau$ . If significant differences were found between control and 4-AP time constants, further experiments could be done to determine whether the change in  $\tau$  stems from the capacitance, or the resistance of the membrane. Experiments such as these could help tease apart the many roles of voltage gated potassium channels in neural signaling.

While most of our results mirrored those from experiments done by other researchers, one parameter that we investigated yielded surprising data. We found that input resistance (R) decreased in the 4-AP condition, as evidenced by a smaller slope than control. In our own experiments, and over several different parameters, 4-AP was shown to cause the excitement of P cells in the leech ganglion. Since R is equal to the slope of an I-V plot, V/I, it is clear that a larger input resistance will require a smaller current to elicit the same membrane voltage. Namely, a cell with larger R will be more excitable. Although it seems likely that 4-AP would increase input resistance, certain studies have shown that it is insensitive to the Kv blocker (Rutecki et al., 1987, Greene et al., 1985). Further investigations on input resistance and 4-AP are necessary because of these seemingly counterintuitive results. The decrease in slope in our experiments might have been due to the fact that each cell was penetrated twice to get control and 4-AP data. After being stressed in the control condition, each cell was expected to generate similar data with 4-AP, however, stress was not controlled for. The cell might have been less likely to fire regardless of 4-AP in the second condition, simply because it had been damaged in the control recordings. To avoid this, we could have remained inside the same cell during the wash-in of 4-AP, followed by a washout to prepare the ganglion for a new recording in a different cell. This would eliminate the problem of multiple penetrations and damage.

The subject of 4-AP and its effect on neural signaling is an interesting one, and the results of these experiments open up several avenues that could be explored. First, Wheeler et al. showed that 4-AP was associated with a slowing at the start of action potentials (1996). This might implicate an effect on the action potential threshold. The postsynaptic effects of 4-AP would also be interesting to investigate. Does blocking of Kv channels cause increased amplitude of post-synaptic potential, and therefore, neruotransmitter release? What is the role of Kv channels in synaptic plasticity, learning and memory? Finally, the behaviour of other cells in the leech ganglion could be investigated in the presence of 4-AP. These could be compared and contrasted to the P cell data obtained in these experiments.

In conclusion, the Kv channels play a significant role in the excitability of neurons. Blocking them using 4-AP causes an increase in the duration and amplitude of action potentials, as well as a slight increase in the time constant, which must be investigated further. Voltage gated potassium channels' role in input resistance is also an interesting avenue that should be pursued.

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# **APPENDIX**

# SAMPLE AVERAGE CALCULATION:

(I-V data, -2nA, control)

 $x_{bar} = \frac{\sum x_i}{N}$  (from i = 1 to i)  $x_{bar} = -87.19 + -84.89 + -87.59$ 

 $x_{bar} = \frac{-259.67}{3}$ 

 $x_{bar} = -86.56 mV$ 

# SAMPLE STANDARD DEVIATION CALCULATION:

(I-V data, -2nA, control)

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

$$SD = \sqrt{\frac{(-87.19 - -86.56)^2 + (-84.89 - -86.56)^2 + (-87.59 - -86.56)^2}{3 - 1}}$$

$$SD = \sqrt{\frac{4.247}{2}}$$

# SD = 2.124

### SAMPLE T-TEST CALCULATION

(input resistance data, control vs. 4-AP)



t = 19.188 > 1.660, and t is significant, p<0.05 (no bonferroni because only one comparison)

## **SAMPLE BONFERRONI CORRECTION:**

alpha level = desired alpha/number tests

alpha level =  $0.05/4 \approx 0.01$ 

## RAW DATA:

P1	I-V (mV	/)					DUR	ATION	(ms)				AMPLI	TUDE	(ms)			
trial	-2nA	-1nA	0(rest)	-2nA	-1nA	0(rest)	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA
1	-88.16	-74.37	-54.19	-47.92	-45.61	-40.11	8.1	8.6	8.6	9	9.5	9.7	50.38	46.9	43.53	58	53.23	49.67
2	-88.63	-71.75	-54.12	-45.16	-42.69	-42.59	8.1	8.2	8.8	9.3	9.5	9.8	49.35	46.33	42.75	58.92	52.39	52.38
3	-85.73	-70.9	-55.15	-47.59	-44.37	-42.02	7.6	8.4	8.4	9.1	9.4	10	50.49	48.55	41.78	59.2	51	50.55
4	-86.79	-73.68	-55.81	-48.02	-43.77	-42.73	8.1	8.1	8.4	9.1	9.6	9.7	51.33	47.52	42.07	57.37	54.06	50.1
5	-86.42	-73.5	-56.1	-45.41	-43.72	-41.25	8	8.5	8.6	9	9.4	10	50.54	46.34	42.78	55.54	50.08	52.53
6	-87.86	-73.84	-55.93	-47.82	-42.03	-40.14	7.9	8.1	8.6	9.4	9.7	9.7	51.8	46.87	43.23	58.35	51.77	49.26
7	-85.62	-72.63	-55.11	-45.42	-45.69	-39.56	7.4	8.6	8.6	9.2	9.6	9.9	51.65	48.11	42.34	55.03	53.42	50.44
8	-86.82	-70.88	-54.93	-44.58	-42.25	-40.39	7.5	8.3	8.5	9.1	9.6	10	51.57	47.09	44.6	55.98	53.29	50.11
9	-85.73	-73.23	-55.54	-47.8	-44.04	-40.23	8.1	8.5	8.6	9	9.6	10	49.59	47.96	41.91	58.13	54.1	53.17
10	-87.1	-71.5	-56.26	-46.44	-43.34	-40.59	7.5	8.5	8.6	9.4	9.5	9.9	51.98	48.74	43.27	54.57	52.75	49.54
11	-86.85	-73.16	-54.65	-47.13	-42.09	-42.28	7.6	8.6	8.8	9.2	9.4	10	52.29	48.94	44.14	59.62	51.82	50.08
12	-86.32	-73.09	-54.94	-44.87	-42.49	-40.98	7.4	8.1	8.5	9.5	9.6	9.8	49.75	48.9	44.12	59.78	51.89	52.96
13	-87.78	-71.78	-54.59	-45.98	-42.25	-42.07	8.1	8.4	8.4	9.3	9.5	9.7	50.3	48.79	42.31	55.8	52.4	50.03
14	-88.41	-74.77	-52.37	-45.19	-42.56	-42.46	7.4	8.6	8.6	9.4	9.7	9.9	49.09	46.04	42.81	59.57	50.23	51.62
15	-87.08	-74.04	-55.06	-46.32	-43.33	-41.18	7.4	8.4	8.4	9.1	9.5	9.9	49.71	46.22	43.54	59.59	54.52	49.32
16	-86.71	-72.44	-54.12	-48.77	-45.76	-41.57	7.5	8.3	8.5	9	9.4	9.7	51.8	49.04	43.04	57.11	53.76	52.22
17	-86.24	-71.38	-55.34	-48.09	-44.61	-42.15	7.6	8.4	8.5	9.1	9.7	9.8	52.33	48.49	42.27	56.34	54.67	51.52
18	-86.76	-72.72	-55.94	-45.77	-43.16	-42.57	7.7	8.1	8.8	9.4	9.7	9.7	50.57	47.74	44.15	57.59	51.85	51.1
19	-87.6	-73.89	-55	-47.64	-44.04	-40.46	7.9	8.1	8.4	9.5	9.6	10	49.2	46.84	42.62	55.39	51.95	52.16
20	-88.72	-72.23	-54.95	-48.42	-42.13	-41.96	8	8.5	8.6	9.1	9.7	10	49.75	48.83	42.16	55.27	51.16	51.86
21	-88.44	-71.97	-52.03	-47.05	-45.46	-42.27	7.9	8.6	8.6	9.3	9.4	9.8	51.12	47.01	42.62	57.1	54.28	53.1
22	-88.1	-71.52	-52.65	-46.69	-42.75	-39.13	7.4	8.3	8.8	9.2	9.7	10	51.08	48.22	41.83	54.14	54.09	52.95
23	-86.61	-73.27	-54.39	-45.83	-42.96	-40.56	7.8	8.5	8.7	9.5	9.5	9.8	49.5	46.99	42.52	55.14	53.59	49.27
24	-87.49	-74.76	-52.8	-46.74	-45.48	-42	7.6	8.2	8.6	9.3	9.6	9.7	50.06	45.83	43.63	56.49	54.98	51.89
25	-85.97	-73.14	-55.88	-44.62	-43.64	-39.01	7.6	8.2	8.7	9.5	9.6	9.9	50.34	48.02	43.44	59.31	50.99	53.17
26	-85.85	-74.56	-55.53	-48.6	-43.48	-42.26	7.9	8.4	8.4	9.3	9.6	9.7	50.88	46.39	43.23	56.5	51.92	52.02
27	-88.04	-70.99	-51.89	-46.94	-43.2	-40	7.4	8.1	8.5	9.5	9.7	9.8	50.29	48.72	43.36	58.75	55.46	49.17
28	-87.39	-74.48	-54.06	-46.07	-43.2	-39.79	7.8	8.3	8.6	9.5	9.5	9.8	48.99	48.96	44.17	56.52	53.86	50.07
29	-88.78	-73.65	-54.07	-47.04	-42.83	-42.21	7.7	8.4	8.8	9.4	9.4	9.7	50.45	45.87	43.24	58.37	52.32	50.12
30	-88.65	-72.99	-56.15	-45.83	-44.3	-42.43	7.5	8.2	8.8	9.2	9.4	9.7	51.1	46.41	42.29	57.37	52.52	52.46
31	-85.56	-74.58	-55.44	-44.39	-45.31	-40.89	7.6	8.4	8.8	9.2	9.6	9.7	52.25	45.77	43.24	55.46	55.58	52.4
32	-87.64	-73.25	-54.92	-45.25	-45.03	-42.22	7.6	8.3	8.6	9.4	9.7	9.7	52.62	47.25	42.67	55.21	52.94	49.6
33	-87.19	-71.92	-53.88	-44.88	-43.69	-39.28	7.4	8.2	8.5	9.2	9.5	10	49.86	48.44	44.29	56.34	50.4	50.17
34	-88.25	-71.84	-55.74	-45.91	-42.49	-39.93	7.5	8.6	8.5	9.2	9.6	10	49.96	45.74	41.75	56.08	55.26	50
35	-86.34	-74.04	-55.15	-46.4	-41.93	-40.26	8.1	8.3	8.6	9	9.7	9.7	49.89	46.88	42.7	55.98	54.52	49.14
mean	-87.19	-72.94	-54.71	-46.47	-43.59	-41.13	7.71	8.35	8.591	9.25	9.56	9.83	50.62	47.45	42.98	57.03	52.94	51.03
SD	0.998	1.1866	1.1808	1.2596	1.1843	1.1508	0.26	0.17	0.134	0.17	0.11	0.13	1.026	1.112	0.787	1.646	1.532	1.378
P2	I-V						DUR	ATION					AMPLI	TUDE				
trial	-2nA	-1nA	0(rest)	-2nA	-1nA	0(rest)	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA
1	-86.12	-73.96	-49.59	-46.4	-40.98	-41.95	7.5	8.4	8.9	9.3	9.7	10.1	45.64	44.75	41.74	55.71	53.66	49.67

2	-84.43	-68.6	-49.88	-45.89	-41.02	-41.26	8	8.6	9	9.2	9.5	10.4	50.29	45.5	41.35	53.79	50.12	50.08
3	-85.91	-68.13	-52.03	-44.23	-42.79	-39.41	7.5	8.4	8.5	8.9	9.2	10.1	50.73	47.85	44.29	56.17	51.46	48.81
4	-85.62	-74.02	-51.33	-44.12	-40.11	-41.17	7.5	8.4	8.8	8.8	9.2	10.4	50.42	45.28	42.71	55.1	53.59	47.92
5	-85.67	-68.94	-48.38	-45.74	-42.84	-39.87	7.9	8.5	8.5	9.3	9.4	10.4	51.63	45.34	43.08	55.74	53.65	51.46
6	-85.74	-68.4	-49.95	-45.83	-40	-38.87	7.9	8.3	8.5	9.3	9.3	10	50.78	47.57	40.97	55.54	50.22	47.98
7	-86.09	-73.24	-50.19	-45.35	-41.68	-41.92	7.7	8.8	8.5	9.4	9.7	10.2	46.45	45.24	42.77	53.56	53.24	52.31
8	-84.75	-71.79	-51.84	-44.51	-43.41	-42.42	7.8	8.4	8.9	9.3	9.6	10.1	51.51	45.32	44.29	55.59	53.03	49.34
9	-83.35	-68.36	-51.26	-44.87	-40.75	-42.59	7.8	8.5	8.7	9.1	9.7	10.3	46.87	47.29	44.21	54.95	50.9	48.71
10	-83.6	-73.18	-50.22	-44.31	-43.02	-41.6	8.1	8.6	8.7	8.8	9.6	10	49.98	45.18	43.75	54.98	52.01	51.24
11	-85.24	-71.26	-50.19	-45.22	-43.49	-38.77	7.6	8.3	8.9	9.1	9.6	10.1	47.86	47.11	44.46	53.27	50.48	50.91
12	-83.91	-68.13	-49.7	-41.96	-40.54	-41.63	7.5	8.6	8.8	9.1	9.2	10.4	45.34	44.89	44	56.79	50.92	48.63
13	-84.67	-71.28	-50.4	-44.49	-42.81	-39.61	8	8.7	8.8	9.2	9.6	10.2	47.67	45.63	42.33	56.67	52.95	53.13
14	-85.24	-72.09	-49.3	-42.11	-43.46	-42.21	7.7	8.4	8.8	9.3	9.4	10.3	47.81	45.14	42.35	55.06	52.72	48.98
15	-83.6	-74.21	-51.34	-43.62	-42.02	-41.22	7.9	8.7	8.9	9.2	9.3	10.1	51.78	46.17	44.5	55.23	50.5	50.95
16	-84.49	-68.27	-49.24	-44.44	-42.44	-39.37	8	8.5	8.7	8.8	9.5	9.9	49.29	46.5	41.56	53.44	51.79	49.22
17	-85.9	-69.12	-51.99	-44.96	-40.73	-40.76	8.1	8.8	8.8	9.3	9.7	10	50.21	46.58	44.22	56.31	52.89	51.41
18	-86.11	-70.79	-50.03	-45.68	-41.05	-38.66	7.9	8.8	8.7	9.3	9.7	10	47.17	46.92	41.69	53.77	51.16	49.01
19	-83.27	-70.96	-50.55	-44.34	-40.7	-41.54	7.7	8.8	8.9	8.9	9.5	10.4	51.76	46.07	43.52	53.69	53.88	51.68
20	-83.63	-69.6	-50.7	-43.78	-42.7	-42.02	8	8.3	9	8.8	9.3	10	46.43	45.78	44.41	53.02	53.06	52.4
21	-85.19	-74.57	-51.06	-43.05	-42.24	-40.29	7.6	8.6	8.7	9	9.6	10.1	50.69	47.48	44.16	53.35	53.23	51.33
22	-83.62	-68.14	-49.22	-44.35	-43.37	-41.55	7.6	8.8	8.5	9.1	9.2	10.3	49.37	45.21	41.89	53.77	53.11	53.2
23	-83.76	-73.33	-49.22	-43.48	-41.05	-38.96	7.6	8.6	8.7	9.1	9.2	10.1	48.97	46.32	41.95	54.85	53.69	52.25
24	-85.34	-71.07	-51.94	-42.35	-40.57	-40.9	8	8.5	9	8.8	9.5	10	49.37	45.18	42.42	56.59	54.44	50.03
25	-84.43	-71.07	-51.12	-43.03	-43.34	-40.35	8.1	8.8	8.7	8.9	9.4	10.3	51.17	44.99	41.85	55.55	50.56	52.82
26	-84.17	-72.38	-51.67	-44.32	-43.22	-40.72	7.8	8.5	8.7	9.3	9.5	9.9	50.54	45.5	41.72	55.97	53.43	51.75
27	-85.08	-70.45	-51.28	-45.26	-40.5	-40.26	7.5	8.8	8.8	9.2	9.2	9.8	49.38	44.83	43.26	53.49	50.57	50.84
28	-85.21	-68.79	-51.69	-42.91	-40.36	-39.83	7.8	8.3	8.9	9.1	9.5	9.8	49.21	45.58	44.12	53.77	52.36	50.46
29	-85.89	-70.94	-51.45	-44.4	-42.61	-41.55	7.8	8.3	8.5	8.8	9.5	10.1	49.24	45.2	43.47	56.92	52.39	49.65
30	-84	-68.15	-48.86	-42.64	-41.84	-42.42	7.8	8.5	8.8	9.3	9.7	10	51.19	45.8	44.34	55.96	52.2	50.1
31	-85.45	-68.97	-48.76	-44.86	-41.73	-41.4	7.5	8.7	8.6	9.3	9.2	10	46.76	45.41	41.62	53.86	52.32	48.31
32	-85.36	-71.2	-51.04	-41.88	-42.55	-41.81	7.9	8.5	8.6	9.2	9.3	10.3	50.46	46.73	42.66	54.28	50.69	49
33	-84.2	-70.91	-49.28	-44.68	-40.76	-39.7	8	8.5	9	9.4	9.7	10.1	47.75	47.46	41.68	56.63	53.13	50.58
34	-86.02	-71.36	-50.76	-45.69	-43.34	-40.06	8	8.7	8.5	9.3	9.4	10.4	51.53	46.39	43.28	56.36	50.89	49.63
35	-86.09	-72.66	-50.07	-43.02	-42.1	-41.6	7.9	8.8	9	8.9	9.5	10	47.18	44.81	42.17	55.45	50.85	50.96
mean	-84.89	-70.81	-50.44	-44.22	-41.89	-40.81	7.8	8.56	8.751	9.12	9.46	10.1	49.21	45.91	42.94	55.01	52.17	50.42
SD	0.9314	2.0326	1.0366	1.2133	1.143	1.158	0.2	0.18	0.169	0.2	0.18	0.18	1.903	0.912	1.117	1.212	1.269	1.493
P3	I-V						DUR/	ATION					AMPLI	TUDE				
trial	-2nA	-1nA	0(rest)	-2nA	-1nA	0(rest)	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA
1	-90.06	-69.34	-48.95	-30.76	-25.51	-26.67	7.5	8.3	8.6	8.7	9.4	9.9	50.93	48.22	45.11	56.58	54.65	52.67
2	-86.26	-75	-51.21	-32.41	-27.53	-29.48	8.6	8.7	8.6	9	9.7	10.3	49.9	48.81	44.67	54.18	55.02	48.58
3	-86.58	-76.98	-50.15	-32.11	-25.53	-29.24	7.5	8.6	8.8	9.4	9.4	9.9	50.6	47.18	45.19	55.99	55.01	51.75
4	-89.61	-72.82	-50.39	-31.97	-25.53	-27.17	8.1	8.7	8.7	8.7	9.7	10	50.54	47.72	43.13	56.34	54.04	51.83

5	-85.99	-79.6	-49	-31.41	-27.35	-27.06	8.5	8.3	8.8	8.8	9.6	10	50.49	49.47	44.97	55.61	55.22	52.1
6	-88.54	-79.76	-50	-31.25	-27.04	-29.07	8.3	8.5	8.8	8.9	9.5	9.9	50.01	49.39	44.67	55.41	54.64	49.3
7	-86.6	-70.84	-49	-31.27	-27.43	-27.43	7.5	8.5	8.8	9.1	9.5	10.2	50.1	48.91	47.21	56.18	54.97	49.12
8	-88.97	-72.26	-51.12	-32.17	-25.59	-29.14	7.8	8.6	8.6	8.9	9.7	9.9	50.22	48.38	46.01	53.86	54.22	50.94
9	-88.04	-67.75	-50.87	-32.19	-26.04	-27.53	7.8	8.3	8.8	9.1	9.5	10.3	50.92	48.97	43.38	53.9	54.81	51.89
10	-85.3	-73.31	-49.6	-31.11	-25.95	-27.45	8.3	8.4	8.6	9.2	9.6	10.3	50.13	47.49	45.52	54.15	55.08	50.79
11	-88.63	-67.59	-50.55	-31.62	-25.74	-27.42	7.8	8.4	8.8	9.1	9.7	10.2	50.47	49.73	46.59	56.32	55.43	50.16
12	-86.76	-79.34	-50.75	-31.4	-26.7	-28.73	8.2	8.5	8.6	9.1	9.6	10.1	50.5	48.07	42.46	53.86	54.41	49.56
13	-85.24	-72.71	-50.5	-31.76	-27.11	-26.96	7.8	8.6	8.7	9.3	9.7	10	49.92	47.32	44.36	55.41	54.67	49.98
14	-88.15	-77.23	-49.2	-31.99	-26.92	-28.44	7.6	8.3	8.6	9.1	9.5	10.1	50.57	48.49	44.86	54.88	53.96	51.72
15	-88.37	-69.76	-50.36	-30.94	-25.58	-27.93	8	8.5	8.8	9.2	9.3	10.2	50.72	47.61	45.11	54.64	53.91	51.74
16	-85.04	-70.73	-49.9	-31.36	-25.97	-27.72	7.9	8.7	8.7	9.3	9.7	10	50.16	47.44	43.99	53.5	54.37	51.38
17	-85.38	-72.28	-49.48	-31.05	-25.74	-28.58	8.6	8.7	8.6	9	9.3	10	50.68	48.63	44.56	54.78	54.44	50.14
18	-86.8	-74.07	-49.05	-31.62	-26.94	-26.86	7.8	8.3	8.6	9.4	9.6	10.2	50.01	49.68	44.87	55.14	55.13	48.95
19	-88.5	-67.45	-50.86	-30.82	-27.05	-29.22	8.6	8.7	8.6	8.7	9.6	10	50.66	49.99	42.68	54.42	54.93	51.44
20	-88.36	-77.99	-50.11	-30.79	-27.05	-29.06	8.2	8.4	8.6	8.9	9.4	10.3	50.58	47.15	46.34	54.9	55.36	51.64
21	-87.03	-72.06	-49.77	-30.91	-27.28	-28.19	8.3	8.5	8.6	9.4	9.7	10.1	50.71	48.95	45.69	55.8	54.04	51.87
22	-89.01	-71.55	-49.15	-31.68	-26.54	-27.26	8.1	8.4	8.8	9.4	9.4	10.1	49.99	49.47	42.05	54.49	54.38	49.36
23	-86.9	-75.9	-50.81	-30.83	-27	-26.78	8.1	8.5	8.6	9	9.5	10.1	50.09	48.76	46.23	56.69	54.63	49.61
24	-85.65	-73.14	-49.24	-32.28	-25.92	-27.47	8.5	8.5	8.6	9.3	9.3	10	50.83	49.79	44.89	55.55	54.5	51.37
25	-88.67	-71.79	-50.43	-31.6	-26.01	-27.87	7.9	8.3	8.6	8.7	9.4	9.9	50.9	48.45	45.54	53.47	55.17	49.46
26	-89.55	-71.72	-49.31	-31.81	-26	-28.88	8.5	8.5	8.7	9.1	9.5	10.2	50.09	47.85	47.34	55.8	54.84	49.83
27	-87.52	-65.79	-49.33	-31.85	-25.8	-27.74	8.1	8.7	8.6	9.4	9.6	10.3	50.13	48.64	44.66	54.04	54.88	52.36
28	-87.46	-67.05	-49.01	-31.46	-27.53	-28.21	8.4	8.7	8.6	9	9.6	10.3	50.45	49.72	46.85	54.19	54.42	51.38
29	-89.27	-71.7	-50.43	-31.32	-27.24	-27.34	7.8	8.3	8.7	8.9	9.4	9.9	50	49.78	45.18	55.18	55.4	49.77
30	-86.88	-71.65	-49.68	-30.81	-26.83	-27.14	7.8	8.5	8.8	8.8	9.3	9.9	50.67	49.33	45.72	54.45	54.67	49.44
31	-87.48	-73.16	-50.38	-32.23	-25.62	-27.58	7.7	8.6	8.7	9.4	9.4	10	50.72	47.49	47.37	54.91	54.07	49.61
32	-89.94	-73.67	-49.72	-32.37	-26.59	-28	8.1	8.5	8.8	9.3	9.6	10	49.91	49.23	42.46	54.95	54.86	50.92
33	-88.39	-75.04	-51	-31.99	-27.06	-27.91	7.7	8.5	8.8	9.4	9.6	10.3	50.4	48.4	46.36	54.91	55	50.39
34	-86.08	-78.36	-50.99	-32.32	-26.22	-28.78	8.3	8.6	8.8	9.2	9.4	10.3	50.64	47.28	42.37	55.59	54.54	51.31
35	-88.68	-77.24	-50.77	-32.24	-26.26	-28.78	8.5	8.6	8.7	9.4	9.4	10.1	50.66	48.52	47.49	56.22	54.94	51.52
mean	-87.59	-73.05	-50.03	-31.59	-26.46	-27.97	8.06	8.51	8.689	9.1	9.52	10.1	50.41	48.58	45.03	55.04	54.7	50.68
SD	1.4449	3.728	0.7252	0.5328	0.6837	0.8161	0.34	0.14	0.09	0.24	0.13	0.15	0.327	0.876	1.514	0.906	0.421	1.128
P4	I-V						<b>DUR</b>	ATION	l i				AMPLI	TUDE				
trial	-2nA	-1nA	0(rest)	-2nA	-1nA	0(rest)	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA
1	-56.8	-38.6	-45.3	-7.6	-6.4	-30.2	5.9	3.3	3.6	5.3	3.2	3.1	35.5	31	10.8	32.1	30.9	26.8
2	-57.2	-31.7	-45.3	-10.2	-7.6	-29.6	5.6	3.3	3.3	5.5	3.3	3.1	32.3	32.1	12.9	33.7	32.8	26.4
3	-57	-36.4	-46.4	-8.4	-8.8	-29.7	5.7	4.1	3.1	5.7	3.3	3.2	35	31.3	13.4	33.3	31.9	27.1
4	-56.7	-33.8	-45.6	-7.7	-9.1	-20.4	5.7	4.1	3.6	5.4	3.4	3.1	32.1	31.1	14.1	34.1	32.3	26.1
5	-56.9	-33.7	-44.9	-13.4	-8.5	-24.5	6	3.3	2.9	5.3	3.4	3.2	34.7	30.1	14.1	33.2	30.3	26.6
6	-56.9	-36.6	-43.8	-13.3	-7.6	-29.9	5.7	3.5	3.6	5.6	3.7	3.1	31	33.2	12.2	32.5	29.7	27.1
7	-57.1	-39.1	-43.9	-12.5	-7	-25.7	5.8	4	2.7	5.4	3.8	3.1	26.9	30.8	12.9	33.1	30.1	27.5

8	-57.3	-39.1	-43.2	-8.4	-7.7	-24.4	5.8	3.9	3.4	5.3	3.6	3.1	31.9	30.8	10.9	32.9	27.6	27.1
9	-56.7	-37	-43.2	-11	-9.4	-24.6	6	3.4	3.3	5.6	3.7	3.2	29.6	29.5	14.4	33.3	32.6	26.8
10	-57.1	-37.9	-42.8	-7.9	-8.4	-24.4	5.7	3.7	2.7	5.3	3.2	3.3	27.3	28	11.1	34.7	28.3	27.9
11	-57.2	-31.3	-45.2	-14.2	-7.7	-26.7	5.9	3.7	3.6	5.6	3.8	3.1	27.7	30.2	12.2	32.6	28.7	27.2
12	-57.3	-38.5	-45.4	-10.2	-6.5	-24.7	5.7	3.8	3.3	5.6	3.7	3.2	33.4	27.3	13.8	33.3	33.4	27.9
13	-56.6	-32.3	-42.9	-10.9	-7.7	-23.2	5.7	3.7	3.4	5.6	3.3	3.3	30.9	28.2	13.5	32.4	29.3	28
14	-57.2	-37.8	-44.6	-14.6	-8.5	-23.2	5.9	4.1	3	5.7	3.4	3	31.2	27.5	13.2	33.6	27.1	27.5
15	-57.1	-35.4	-44.8	-10.6	-8.7	-27	5.7	3.6	3.6	5.6	3	2.9	29.8	31.6	11.5	32	32	28.1
16	-57.3	-34.6	-46.1	-14.4	-7	-28.5	5.7	3.5	3.3	5.5	3.6	3.2	32.7	29.7	11.5	33.7	27.7	26
17	-56.8	-33.6	-45.3	-12.2	-7.1	-23	5.8	3.4	3.2	5.7	3.2	2.9	27.9	29.6	12.1	34	31.3	26.7
18	-57	-37.5	-46.6	-9.5	-6.8	-21.9	5.9	3.5	3.4	5.4	3.8	3.2	27.3	29.1	11.8	33.7	31.9	27.2
19	-56.7	-39.1	-45.6	-7.1	-6.4	-28.8	5.9	3.8	2.8	5.5	3.8	2.9	29.7	31.9	12.2	34.1	30.2	26.9
20	-57	-37.9	-45.1	-11.2	-7.6	-27.8	5.5	3.6	3	5.6	3.7	2.9	34.1	31.7	14.3	34.4	32	25.9
21	-57.1	-37.9	-45.9	-12	-6.3	-30.7	5.6	3.8	2.8	5.3	3.1	2.9	29.5	31.6	11.3	34	29.4	28.1
22	-56.8	-38.9	-43.4	-11.8	-8.7	-24.1	6	3.3	3.6	5.3	3.4	3.1	32.5	31.1	11	32.5	31.8	27.5
23	-56.9	-36.3	-46.6	-12.3	-7.4	-26	6	3.6	2.7	5.6	3.4	2.9	33.2	28	13	33.2	28	27.5
24	-57	-36	-46.4	-11.9	-7	-30.1	5.7	3.9	3.5	5.5	3.7	3.3	31.6	30.1	11.2	32.4	29.1	26.3
25	-57	-32.9	-46.4	-10.3	-7.6	-24.6	5.9	3.3	2.7	5.5	3.6	2.9	32.2	32	15.3	32.3	32.2	26.5
26	-57	-38.4	-43.7	-10.6	-9.2	-27.1	5.8	3.7	2.9	5.4	3.6	3.3	34.7	31.5	13	33.5	30.2	26.4
27	-57.3	-38.1	-44.2	-11.1	-7.5	-30.9	5.9	4	3.5	5.6	3.7	2.9	32.7	29.8	14.2	32.1	31	27.7
28	-57.2	-31.3	-43.1	-8.6	-6.7	-25	5.9	3.6	3.5	5.5	3.2	3.1	28.8	32.2	11.2	34	27	27.5
29	-57.3	-38.3	-46.1	-13.3	-7.9	-26	5.9	3.6	2.8	5.7	3.7	3	36.2	32.7	12.4	34.1	30.1	27.6
30	-57.2	-38.6	-45.7	-8.6	-6.6	-23.7	5.6	3.4	3.5	5.5	3.4	2.9	29.8	26.7	14.2	34.6	29.9	26.9
31	-56.6	-34.2	-43	-13.3	-8.1	-20.5	5.6	3.7	2.7	5.4	3.1	3	33.8	33	12.7	32	27.6	27
32	-56.7	-36.3	-42.9	-11.1	-8.4	-30.7	5.6	3.5	3	5.3	3.7	3.3	34.7	27.3	11.1	33.3	32	27.8
33	-56.8	-32.6	-45.9	-9.6	-7.6	-29.3	5.5	3.8	3.5	5.3	3.2	3.3	28.5	27.5	11.1	32.4	33.1	26.8
34	-56.7	-33.9	-43.7	-12.1	-9.1	-22.8	5.8	3.5	3.4	5.5	3.4	3.1	30.9	27.1	13.8	33	28.4	27.8
35	-56.9	-32.7	-43.6	-7.6	-7.3	-25.3	5.9	3.8	2.9	5.6	3.1	2.9	35.8	27.6	12.2	34.3	30.6	26
mean	-55.41	-34.96	-43.52	-10.55	-7.711	-25.42	5.78	3.65	3.194	5.49	3.46	3.09	31.6	30.08	12.59	33.27	30.3	27.09
SD	0.2203	2.5707	1.2587	2.1338	0.8861	3.0269	0.15	0.24	0.332	0.14	0.25	0.15	2.663	1.914	1.252	0.797	1.85	0.644
P5	I-V						DUR	ATION					AMPLI	TUDE				
rial	-2nA	-1nA	0(rest)	-2nA	-1nA	0(rest)	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA	1nA	2nA	3nA
1	-41.7	-39.2	-43	-8	-5.6	-29.9	5.1	3.9	2.9	5	3.9	3.1	74.2	78.7	83.5	80.4	83.4	88.1
2	-37.3	-36.9	-50.3	-5.7	-5.7	-32.2	4.9	3.8	2.5	4.9	3.9	3	80.8	82	91.8	79.9	88.7	88.5
3	-37.6	-25.1	-48.1	-5.4	-5.4	-35.5	4.9	3.6	2.9	4.9	3.6	3	78.4	78.7	91.5	81.3	83.4	87.5
4	-33.9	-28.5	-47.5	-8.8	-6	-32.5	4.8	3.7	2.6	5	4	3.3	81.2	79.9	90.4	78	85	89.5
5	-30.5	-34.5	-42.5	-8.1	-5.4	-29.6	4.9	3.5	2.5	5.2	3.6	3.1	80.2	80.7	90.4	78.3	79.6	89.2
6	-33.8	-38.1	-47.8	-8.1	-6	-35.3	4.8	3.5	2.7	5.3	3.6	3.4	72.5	81.4	93.2	78.4	83.4	89.8
7	-39.6	-30.2	-44.6	-8.3	-5.5	-35.6	4.9	3.6	2.8	5.3	4	3.4	81.1	81.9	90.9	78.6	88.3	89.1
8	-36.1	-28.9	-50.3	-6.8	-5.5	-31.2	5	3.8	2.8	5	3.5	3.2	81.5	85.4	90.7	78.7	79.1	88.4
9	-34.5	-35.3	-45.4	-6.5	-5.8	-30.4	4.8	3.7	2.5	5.3	4.2	3.2	72.2	80.1	88.9	79.5	86.6	90
10	-38.2	-20.7	-46.1	-6.7	-5.6	-33.5	5.1	3.8	2.8	4.9	4	3.3	74.1	84.2	85.1	81.1	81.3	90

11	-31.6	-39.1	-50.1	-8.8	-5.7	-31.8	5.1	3.9	2.9	4.9	3.5	3.3	81.5	86.7	88	78.1	77.7	90.2
12	-32.1	-39.9	-46.5	-7.2	-6	-33.6	5.1	3.6	3	4.9	4.2	3.3	78.5	87.6	86.4	80.8	79.7	88.5
13	-32.1	-25.1	-44.2	-7.8	-5.9	-32.4	4.8	3.6	2.5	5	4.2	3.1	71.8	86	88.9	79.9	84.8	88.4
14	-31.9	-22.1	-43	-5.5	-6	-32	4.8	3.8	2.5	5.1	3.8	3.5	76.2	78.9	84.4	80.3	84.6	90.9
15	-31.9	-29.2	-49.7	-6.4	-5.9	-30.7	4.7	3.9	2.8	5.3	3.9	3.1	71.7	86.8	86.9	80.2	80.3	87.7
16	-40.5	-25.7	-47.5	-5.8	-5.7	-34.5	4.7	3.6	3	5.3	3.8	3.1	81.6	87.8	88.5	81.1	88.2	88.1
17	-36.5	-27.6	-41.5	-7.8	-5.7	-31	5	3.9	2.6	5.3	3.9	3.3	81.1	83.1	91.7	81.6	79.1	89.5
18	-33.1	-27.5	-46.2	-8.6	-5.9	-34.2	4.7	3.8	2.6	5.3	4.2	3.2	79.4	79.7	85.9	81.1	78.3	88.5
19	-35.6	-22.4	-47.6	-5.8	-6	-34.9	4.8	3.8	2.8	5	3.6	3	72.7	79.6	82.9	81.4	86.4	87.5
20	-31.1	-34.4	-46.2	-6.3	-5.4	-30.4	4.9	3.6	2.7	4.8	4.1	3.1	73.3	83.6	92.6	79.7	81	87.6
21	-35.2	-39.1	-42.4	-7.1	-5.6	-35.2	5.1	3.9	2.6	4.8	3.7	3.6	78.5	87.6	86.4	79.8	81.2	88.4
22	-32.5	-26.3	-48.4	-6.1	-6	-32.1	4.7	3.7	2.7	5.2	4	3.6	71.2	85.2	88	79.4	88.9	87.8
23	-41.5	-42.5	-46.9	-7.2	-5.6	-34.1	5.1	3.9	2.6	4.9	3.6	3	75.3	80.7	91.2	77.7	87.9	87.8
24	-35.6	-40.6	-48.6	-8.1	-5.9	-35	5	3.7	3	4.9	4.2	3.2	78.9	86.6	93.3	77.9	84.8	88.4
25	-36.4	-28.8	-42.4	-8.7	-5.7	-33.7	5.1	3.7	3	4.9	4.2	3	76.9	87.8	90.1	80.8	86.4	90.2
26	-40.4	-27.5	-48.8	-6.7	-5.8	-34.8	4.8	3.5	2.9	5.2	3.6	3.3	76.7	87.3	89.4	81.3	85.2	90.9
27	-40.5	-32.5	-48.4	-5.2	-6	-31.9	4.9	3.7	2.9	5	4	3	77.2	79.4	90.1	81.5	77.8	89.5
28	-31./	-35.6	-43.9	-8	-5.9	-34.4	4./	3.9	2.8	5.1	3.8	3.5	/6.9	85.3	91	/8.9	85.8	88.2
29	-33./	-41.3	-45.3	-6.4	-5.4	-30.7	4.8	3.8	2.9	5.1	3.8	3.5	80.7	87.8	87.8	80.2	/8.9	89.7
30	-37.8	-23.5	-43.5	-5.6	-6	-30.7	4.9	3.8	2.7	5.3	4	3.2	/3.1	86.3	87	/9.5	81.6	87.4
31	-34.4	-20.8	-46.4	-7.3	-5.9	-32.5	5	3.9	2.8	5.3	3./	3.1	74.1	85.1	86.7	80.9	84.6	89.3
32	-39.5	-27.3	-44.1	-5.3	-5.4	-30.8	4.8	3./	2.7	5	3./	3.6	74.5	18	90.3	81.5	82.5	88.9
33	-40	-37.9	-43.4	-8.2	-5.6	-33.9	4.9	3./	2.9	5.1	3.8	3.5	//.1	80.7	94.1	//.9	83.2	87.5
34	-32.1	-38.9	-42.8	-6.6	-5.4	-34.9	4./	3.9	3	5.2	3.6	3	80.5	/9.9	93	81.5	/9./	8/./
35	-41.9	-22.6	-47.2	-6.4	-5./	-30	4.8	3.5	2.6	5.3	4.1	3.6	/1.6	84	86.9	78.9	82.3	87.8
mean	-34.81	-30.44	-44./4	-6.819	-5./31	-31.83	4.89	3.73	2.757	5.09	3.8/	3.25	76.78	83.36	89.08	79.89	83.13	88.76
SD	3.5239	6.7409	2.5/13	1.1197	0.218	1.8912	0.14	0.13	0.16/	0.1/	0.23	0.2	3.518	3.213	2.893	1.266	3.381	1.022

## TAU DATA

-2nA		cell 1		cell2	(	ell3			
time point	time(ms)	ΔV	ln(ΔV/e)	ΔV	$ln(\Delta V/e)$	VV	ln(ΔV/e)	ave In(ΔV/e)	SD
	1 0	34.38	2.537475	37.83	2.633102439	41.99	2.737431495	2.636002978	0.100009798
÷	2 11.1	17.53	1.863913699	16.52	1.804571768	17.85	1.882003508	1.850162992	0.040505926
	3 16.3	9.64	1.265921109	11.12	1.408745289	9.54	1.255493485	1.310053294	0.085628653
· · · · · · · · · · · · · · · · · · ·	4 35.4	8.12	1.094330154	8.11	1.093097868	6.73	0.906575144	1.031334389	0.108046432
	5 47.6	7.32	0.990610328	5.13	0.635105659	4.88	0.58514522	0.736953736	0.221088809
	6 69.5	3.32	0.199964783	4.39	0.479329227	2.62	-0.036825682	0.214156109	0.258369925
	7 98.8	1.89	-0.363423171	1.27	-0.7609831	0.67	-1.400477567	-0.841627946	0.523209469
1	8 124.9	1.02	-0.980197373	0.79	-1.235722334	0.43	-1.84397007	-1.353296592	0.443726941
	1 0	8.43	1.131796772	4.49	0.501852702	4.12	0.415853163	0.683167546	0.390896556
	2 11.1	7.97	1.075684493	3.64	0.291983682	3.68	0.302912752	0.556860309	0.449348152
	3 16.3	5.32	0.671473303	2.58	-0.052210601	2.98	0.091923301	0.237062001	0.383051602
	4 35.4	3.64	0.291983682	2.12	-0.248583911	2.32	-0.158432814	-0.038344348	0.2896019
	5 47.6	2.57	-0.056094101	1.55	-0.561745069	1.67	-0.487176374	-0.368338515	0.272969875
	6 69.5	1.67	-0.487176374	1.02	-0.980197373	1.13	-0.877782367	-0.781718705	0.260170323
	7 98.8	1.21	-0.80937964	0.48	-1.733969175	0.76	-1.274436846	-1.27259522	0.462297518
;	8 124.9	0.38	-1.967584026	0.37	-1.994252273	0.49	-1.713349888	-1.891728729	0.155055013



## I-V DATA



### **DURATION AND AMPLITUDE DATA**

DURATION				AMPLITUDE				= CONT
	1nA	2nA	3nA		1nA	2nA	3nA	
total mean	6.84743	6.561142857	6.396571429	total mean	51.72394286	51.07685714	46.52325714	_
	7.61029	7.173714286	7.278857143	3	56.04502857	54.65114286	53.59702857	= 4-AP
total SD	1.29618	2.356375274	2.811713944	total SD	14.64022697	17.62969608	24.58244127	
	1.9151	2.882586528	3.372617739		14.85717199	16.97052427	19.90942601	

