Biowave Corporation – Rabbit Experiment

Test Report # 1-030410

The Experiment:

The neurostimulation experiment was performed in an attempt to quantify the distribution of the electric field and currents as a function of the frequency components for a Biowave neurostimulator. Additionally, the threshold for applied field strength necessary to place a muscle group in tension was determined for both surface electrodes and subcutaneous (minimally invasive) electrodes. No experiments as to pain or pain control were performed. Only one rabbit experiment was performed. Both statistically and for reporting purposes the observations reported herein can only be considered anecdotal.

Animal:

A 4.5 kg male New Zealand White Rabbit was used as the test subject. Prior approval for the experiment was obtained from the proper institutional panel and all USDA guidelines as to the humane care of research animals was rigorously followed. The animal used was part of an established heart failure protocol and served as a normal control for that protocol. Dr. Carter is one of the investigators under that protocol. The animal's age at the time of the experiment was approximately 3 years old. The animal was clean, well nourished, hydrated and healthy at the time of the experiment. This experiment was scheduled to coincide with the collection of a terminal echocardiographic study and organ harvest as outlined in the predicate protocol. For the echo and electro-stimulation experimental procedures the animal was maintained in an unconscious state with chemical anesthesia (xylazine [for induction] and ketamine [for induction and maintenance] via IM injection). The echo study was performed before the electro-stimulation experiment to assure collection of the cardiac measurements in the event the animal died during the electro-stimulation experiment.

Experimental Preparation:

The animal was shaved continuously from ventral to dorsal aspect (stomach to back) at a major axis position corresponding to approximately the middle of the abdomen. This region was chosen for ease of surgical preparation and the distance from the heart (approximately 15 cm). The skin was additionally prepared with a depilatory to assure a clean skin surface. Access to subcutaneous structures was made through small ports (0.5 cm long) incised into the skin and blunt dissected to muscle. The ports were prepared using accepted surgical practices. Prior to the procedure the port regions were infused with 2% lidocaine solution to assure that the animal experienced no discomfort. 14-gauge angiocaths with insulated sheaths were used as the two electrodes and were placed into the two sites corresponding to the opposing surfaces or sides of the body. A pre-summed alternating current high frequency signal composed of 8.000 kHz and 8.122 kHz sine waves was alternately introduced into both of the opposing electrodes. The ports around these were cleaned and closed with 2-0 silk. Two 1-inch diameter round surface electrodes were placed near the sites of the 14-gauge electrodes. Two 20-gauge angiocaths with insulating sheaths were placed in two of the ports along the line separating the two stimulation sites. These ports were also closed. The angiocaths were held in place with Micropore wound tape. The port at the midpoint between the two stimulating sites was used to introduce a small induction coil similar to those used to measure blood flow. This coil was fully insulated electrically. The three measurement port signals were amplified with a home built low noise differential amplifier and the data was recorded with a digital oscilloscope. Additionally, stimulation current was measured as a differential voltage across a shunt resistor placed in series with the feed electrode wire. This signal was also collected.

Results:

The difference between the electrical potential and in turn the electric field intensity required to place a muscle group in tension, as a function of electrode type, was large. It required about 9 VAC rms to place the animal's muscles (along the electrode path) in tension when surface electrodes were used. For the subcutaneous electrodes (angiocaths) less than 4 VAC rms was required. This observation is consistent with the lower impedance and losses associated with signal injection into an ion-rich medium as opposed to the additional voltage required to compensate for the voltage drop across the skin surface. As a result of this impedance difference the amount of current was much higher (about a factor of 5) for the subcutaneous electrode at a given electrode potential.

The signal from the induction coil was noisy and quite small. However, FFT spectral decomposition (to determine the presence and magnitude of the individual component frequencies that make up the total signal) of this signal showed a current of 60 microamperes at a frequency equal to the difference between the components of the pre-summed 8.000 and 8.122 kHz high frequency signals, or 122 Hz. This current is more than sufficient, and is centered within the physiologically active frequency region, to affect ion flow and thus interfere with electrochemical reactions or signaling within the body. There were also some weak signals present that corresponded to the first and second harmonic of the difference frequency (244 Hz and 366 Hz). Additionally, there were very large high frequency components that occurred at 16.122 kHz, 8.0 kHz and 8.122 kHz which correspond to the summation of the high frequency signals and to each individual high frequency signal which originated from the Biowave neurostimulator. This was expected since the electronic mixing process within a mammal is probably not very efficient. The 20-gauge electrodes showed attenuated signals corresponding to the Biowave high frequency signals. However, the amplitude of these signals was larger, for a given electrode potential, when the 14-gauge angiocaths were used. This is consistent with the lower electrode/body interface impedance seen by the subcutaneous electrodes.

The resulting components measured indicate that a further multiplication of the pre-summed high frequency signals (Fourier Transform) occurred inside the body which suggests that polarized tissues inside the body have a nonlinear characteristic to them which would be required in order to cause a multiplication of the two high frequency sine waves.

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