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EC NEUROLOGY
Research Article

Factors Affecting Stimulus Artifact: Solution Factors

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Received: February 12, 2017; **Published:** March 13, 2017

Abstract

Stimulus artifact is a common problem in neurophysiological testing and much work has been done to find ways to remove it from EEG, evoked potential, and nerve conduction study recordings. The purpose of this paper is to demonstrate how changes in the physical properties of the solution in which electrodes are embedded (i.e., body tissue) affect stimulus artifact. This is accomplished by varying the concentrations of glucose, saline, insulin and oxygen in solution and recording the resultant changes in stimulus artifact. These changes in stimulus artifact are t hen related to changes in three physical properties describing the solution (viscosity, index of refraction and impedance). Increasing concentrations of glucose increased both the viscosity and the electrical impedance of the solution. With constant current stimulation, this leads to an increase in stimulus artifact. Increased salt concentrations showed a greater effect on solution impedance than glucose and hence, a greater increase in stimulation artifact. The presence of oxygen in a solution also modified stimulation artifact, likely due to chemical reactions at the surface of stainless steel electrodes and the solution.

In summary, stimulus artifact is not just a nuisance that requires technical troubleshooting or elimination from neurophysiological recordings. When present, it can provide important insight and information regarding changes in body tissues in which the stimulating electrodes are embedded.

Keywords: *Stimulus Artifact; Neurophysiological Testing; Glucose*

Introduction

During neurophysiological recordings, artifact generated by the stimulation process is frequently noted. It interferes with clinical recordings particularly when the distance between the stimulating and recording electrodes is small, the stimulus duration is long, or the stimulus voltage is high [1]. There is also evidence that stimulus artifact can distort the signal, producing a prolonged "tail," which overlaps and obscures the neural response [2]. There has been very little research on the properties of stimulation artifact which might assist a clinician in predicting when it may occur to proactively take action to minimize or eliminate it. A Medline search for the term "stimulus artifact" revealed less than 200 references. Of these references, a large number contained the terms removal, rejection, suppression, elimination, or compensation and related terms, suggesting that the main objective of the articles was to find methods of abolishing stimulus artifact [3-5]. However, no algorithm can truly remove stimulus artifact during a recording if physical and biological factors that directly impact the artifact are not accounted for. It is, thus, important to understand the factors that may affect stimulus artifact. To this end, one purpose of this paper will be to outline some effects that properties of a solution in which electrodes are placed (i.e., body tissues) may have on stimulus artifact.

Three papers by Kent [6], Hua [2] and Hamming [7] focus on the etiology and factors contributing to stimulus artifact. Kent [6] created a finite element model describing the tissue near an electrode in order to predict changes in the stimulus artifact as scar tissue formed

around the electrode. Hua [2] created an empirical model of the medium in which electrodes are placed as a set of resistors and capacitors, and showed that electrical properties of the medium substantially affects stimulus artifact, especially the slow decay of the signal (the long "tail"). Although empirical modeling is important, a clear theoretical understanding of how stimulus artifact is modified by specific properties of a solution would be even more valuable. Hamming and Lovely [7] found that impedance characteristics of recording electrodes do not affect the magnitude of stimulus artifact, but their study did support Hua's findings that biologic changes in the nerve, hence inter-subject, contributed the greatest variation to stimulation artifact [7]. Since it is relatively common to see changes in glucose and saline levels in patients, this paper will initially concentrate on the effects of these variables on stimulus artifact. This paper will also study the effect of variations in oxygen concentration on stimulus artifact because patient tissue oxygenation may vary during recordings and stainless steel electrodes are often used for recording and stimulation. Stainless steel is an iron alloy, and there are very strong chemical reactions between iron and oxygen. Finally, given the fact that highly charged proteins may interact with stimulating electrodes in a very different way than the small anions and cations present in saline, the effects of one charged molecule commonly found in patients, insulin, will be investigated.

Methods

Solutions

One solution used extensively in nerve conduction *in-vitro* studies is artificial Cerebrospinal Fluid (aCSF) [8] [110.2 millimolar (mM) NaCl, 4 mM $MgSO_4$, 3.9 mM KCl, 17.8 mM $NaHCO_3$, 3 mM KH_2PO_4, 1.2 mM CaCl, and 10 mM HEPES]. To investigate the effect of glucose on stimulation artifact, varying glucose concentrations were added to this base solution. The concentrations of glucose used were 0 mM, 1 mM, 5 mM, 10 mM, 50 mM, 100 mM, 250 mM, 500 mM, 750 mM, and 1000 mM. Although only glucoses in the range of 1 mM to 55 mM might be encountered in clinical situations, larger glucose concentrations were studied in order to elicit the type of effect(s) that increased levels may have on stimulus artifact. Even though effects may be small in the clinical range, they may be clinically important if the stimulus artifact at these low concentrations is much larger than the neural response. The effects of insulin on stimulation artifact were studied at concentrations of 100 nanomolar (nM) and 1000 nM (roughly 1,000 to 10,000 times the normal blood level), added to aCSF with 5.55 mM of glucose (normal blood concentration). In testing the effect of dissolved oxygen, pure oxygen was vigorously bubbled through aCSF with 5.55 mM of glucose before being used. The deoxygenated solution was the same solution through which pure nitrogen gas was vigorously bubbled prior to use.

Saline solutions were created by adding NaCl to distilled water in order to achieve NaCl concentrations in the range of 0 mM to 200 mM. The temperature during all recordings was roughly 21°C (69.8ºF).

Testing Physical Properties of the Solution

Three physical properties of each solution were measured: viscosity, index of refraction, and impedance. Viscosity is a measure of a fluid's resistance to flow which is inversely related to the ability of molecules in the solution to diffuse from one point to another. This was measured using a calibrated Ubbelohde viscometer (Model-0C, Cannon Instrument Company, State College, PA). The index of refraction is a measure of how strongly a solution bends light. It is very dependent on the presence of molecules in the solution that change the way the solution responds to an electric field. This was measured using a hand-held refractometer (RHC- 200ATC, C&A Scientific, Manassas VA).

Measuring solution impedance is complex because it depends on the total current density, the electrodes used, and the frequency of the stimulation current. Reproducible responses were difficult to obtain using very low currents or DC stimulation. The most reproducible results were obtained using a sinusoidal constant voltage stimulus at 1 Volt and 500 Hz. The root mean square (RMS) voltage (the average of the square of the voltage) across the electrodes and the RMS current flowing through them were measured using 2 Hewlett-Packard 3457a multimeters (Agilent, Santa Clara, CA). The impedance was then determined using Ohm's law (I = V/R).

Electrodes were made from thin pieces of stainless steel in the shape of a rectangle with a surface area of 5.25 cm^2. Each test was performed with the electrodes submerged in exactly 15 ml of fluid so that the geometric relationships between the electrodes and the solution remained constant.

Recordings

A piece of approximately 9-inch tubing, containing 3 stainless steel needle (stimulating) electrodes (Rhythmlink, Columbia SC), was arranged in a tripolar configuration, and was connected to a Grass Stimulator (S88, West Warwick MA) using a Grass PSIU6 constant current stimulus isolation unit. The stimulus consisted of unipolar pulses with a duration of 0.03 ms presented 5 times/second. Bipolar recordings were made with stainless steel recording electrodes and amplified using a Grass Model 12 amplifier (Grass-Astromed, West Warwick, RI) with a low frequency filter of 0.3 Hz and a high frequency filter of 20 kHz. The signals were digitized at 240 kHz using a National Instruments USB-6210 A/D convertor (National Instruments, Austin, TX), averaged 20 times, and stored every 4 seconds.

Abstracted Parameters

From each tracing, the peak-to-peak amplitude of the stimulus artifact waveform was measured. In addition, the time it takes for the voltage of the stimulus artifact to drop to half its value at 0.15 ms following stimulus is also computed. This is called the decay time.

Statistics

Spearman rank correlations were used as the primary measure to determine whether there was a statistically significant relationship between an abstracted parameter, one of the physical parameters, and concentration. Linear regression analyses (Statistica, Statsoft Tulsa OK) are used to determine the relationships between different parameters of the stimulus artifact and the physical properties of the solution. Because the lowest order effects of any physical perturbation are often linear, linear regression analysis allows prediction of the effect of a physical factor on stimulus artifact at concentrations that were not explicitly studied.

Results

Effect of Glucose Concentration

Figure 1 shows stimulus artifact waveform traces as a function of glucose concentration in aCSF. Although the shape of the waveform does not change with glucose concentration, the peak to peak stimulus artifact voltage does. Figure 2 shows the dependence of the peak to peak stimulus artifact on glucose concentration and solution impedance. Figure 3 demonstrates that physical attributes of the glucose solutions, including viscosity (Spearman $R = 0.94$ $p < 0.001$), impedance (Spearman $R = 0.95$, $p < 0.001$), and index of refraction (Spearman $R = 1.0$), increase monotonically with glucose concentration. Figure 2 shows that the peak-to-peak value of stimulus artifact amplitude also increases monotonically with glucose concentration (Spearman $R = 0.89$, $p < 0.001$) and is linearly related to the impedance measure. Linear regression analysis shows that the peak-to-peak amplitude of stimulus artifact increases by 2.1 μV/mM glucose [standard error (SE) = 0.08 $p < 0.001$] or by 34.1 μV/Ohm (SE = 0.75, $p < 0.001$). On a relative basis, stimulus artifact increases by 0.11% / mM glucose (SE = 0.0035%), or by 180% for every (mm^2/sec) change in viscosity (SE = 4%, $p < 0.001$). In terms of the index of refraction, stimulus artifact increases by 41% (SE = 1%, $p < 0.001$) for every 0.01 unit change in index of refraction.

The peak-to-peak amplitude beyond 0.15 ms is also significantly related to glucose concentration (Spearman $R = 0.78$, $p < 0.001$) while the peak-to-peak amplitude beyond 0.3 ms is not related to glucose concentration (Spearman $R = 0.29$, $p > 0.05$). The decay time is not dependent on glucose concentration (Spearman $R = -0.12$, $p > 0.05$).

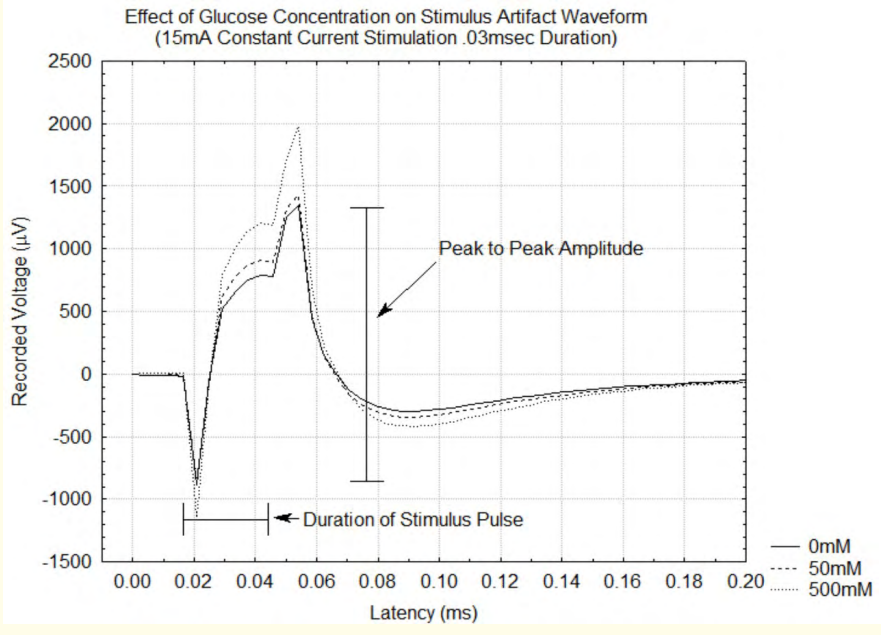

Figure 1: *The graph on the left shows the effect of increasing glucose concentration on the stimulus artifact tracing. The bar at the bottom of the graph indicates the time during which the constant current stimulus is being delivered (i.e., duration of the stimulation pulse).*

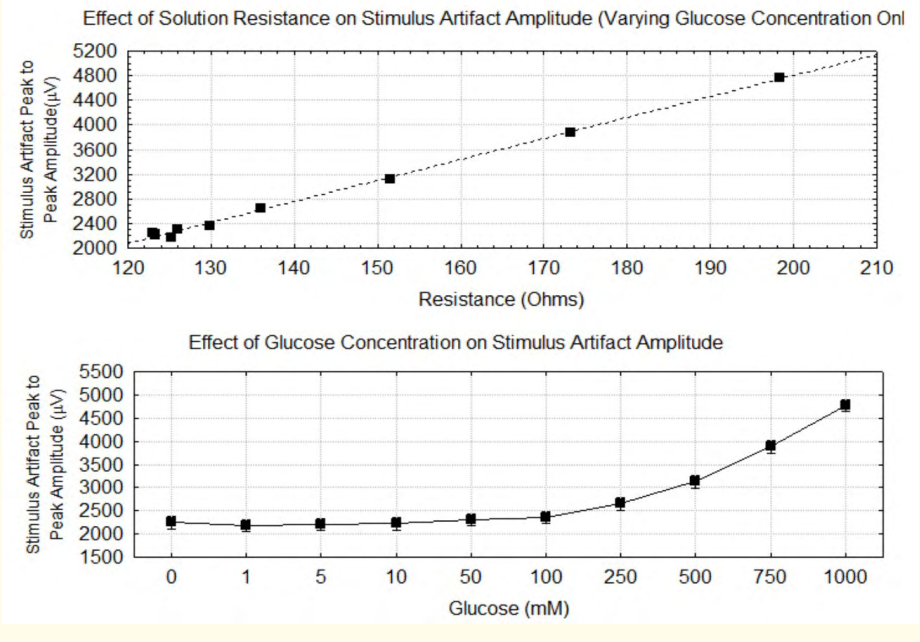

Figure 2: *The graphs on the right show how the amplitude of the stimulus artifact changes with glucose concentration and solution impedances.*

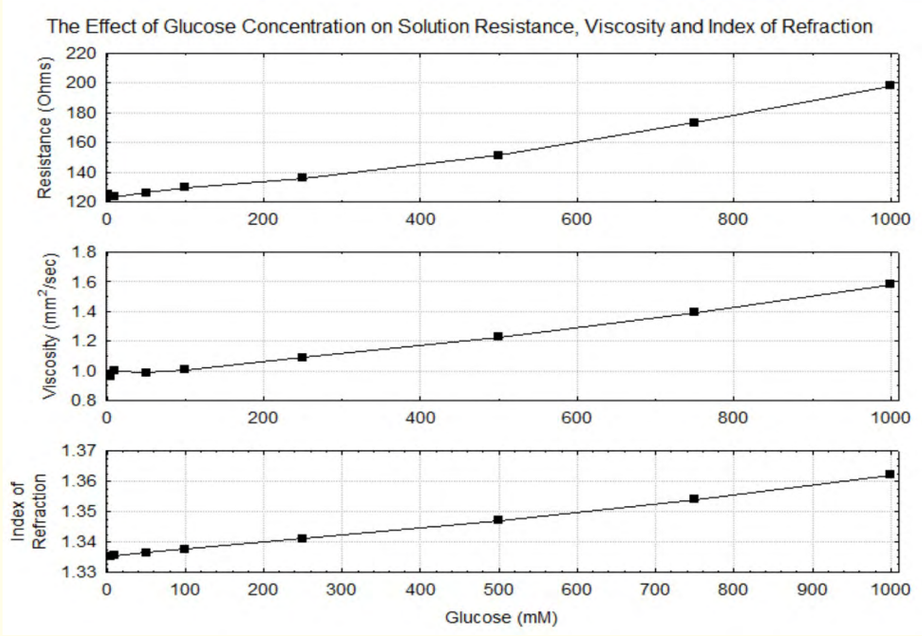

Figure 3: *Correlation of the physical properties of the solution (viscosity, index of refraction and impedance) with increasing glucose concentrations.*

Effect of NaCl Concentration

When the impedance of the solution is high, the voltage required to produce constant current stimulus exceeds the maximum capability of the PSIU6 unit; therefore, the entire range of NaCl concentrations could not be explored using the same stimulus current. When very low currents are used in low impedance conditions, stimulus artifact is of very low amplitude and the tracing is noisy. Figure 4 shows changes in the waveform as a function of NaCl concentration when 15 mA current is used. It is clear that both the shape and amplitude of the stimulus artifact change with salt concentration. Figure 5 is a corresponding figure using 15 μA stimulus current. Even at the lower stimulus current, there are corresponding changes in both the waveform and amplitude with changes in salt concentration. With 15 mA current, figure 5a shows that stimulus artifact amplitude declines with increasing salt concentration (Spearman R = -0.98, $p < 0.001$).

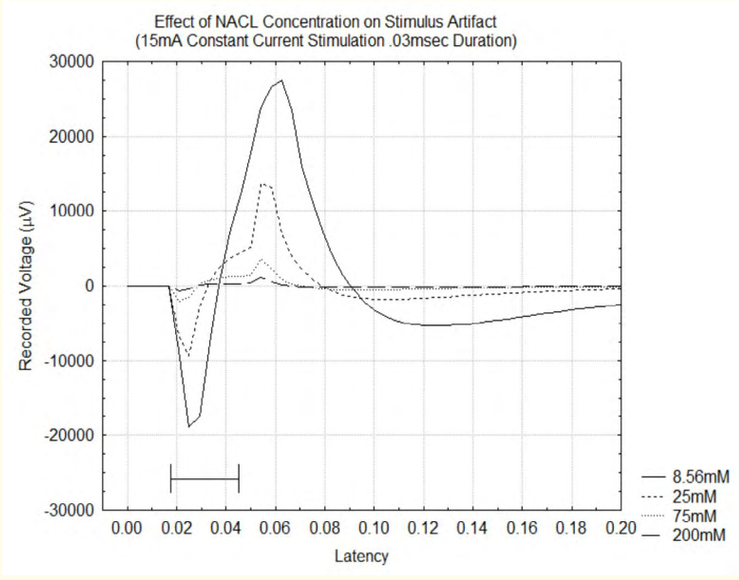

Figure 4: *The effect of NaCl concentration on the stimulus artifact waveform when a 15 mA stimulus current is applied. The bar at the bottom indicates the duration of the stimulus. The amplitude of the stimulus artifact declines with increasing salt concentration.*

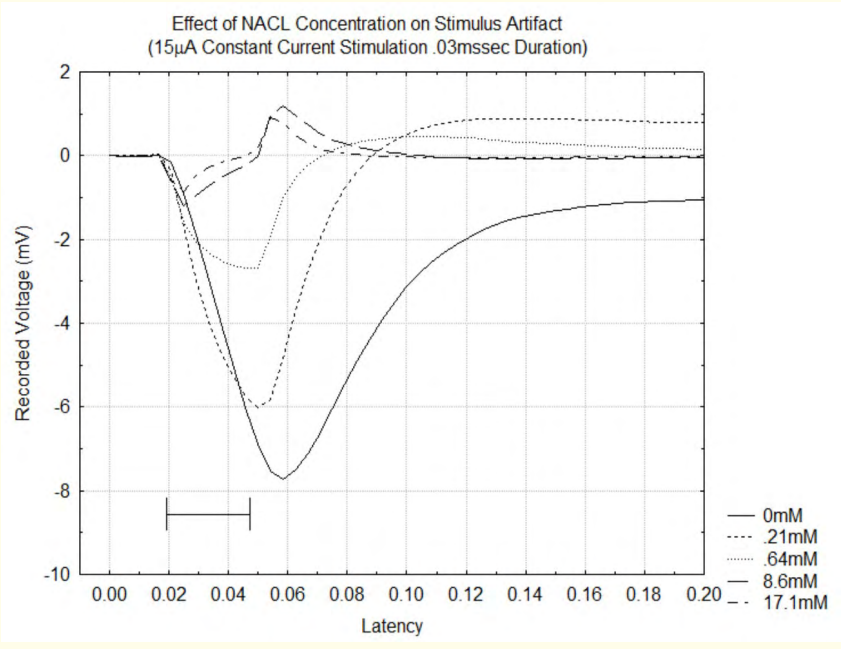

Figure 5: *Effect of NaCl concentration on stimulus artifact when a 15 μA stimulus current is applied. Demonstrates the dramatic changes in waveform with changes in salt concentration and the marked increase in duration of stimulus artifact with low salt concentrations. The bar at the bottom of the graph indicates the duration of the stimulation.*

Similarly, stimulus artifact amplitude beyond 0.15 ms (Spearman R = -0.99, $p < 0.001$) and 0.3 ms (Spearman R = -0.96, $p < 0.001$) both decline with increasing salt concentration. Figure 5b shows that, unlike with increasing glucose concentration, the decay time of stimulus artifact declines with increasing salt concentration (Spearman R = -0.53, $p < 0.05$) even though the saline solutions show little variation in viscosity or index of refraction. Figure 6c demonstrates that the relationship between stimulus artifact and impedance with 15 mA current stimulus is linear only when the solution impedance is low. In this linear region, the peak-to-peak amplitude increases by 43 μV/Ohm (SE = 0.75, $p < 0.001$), a number that is similar to that seen with the glucose solutions. Figure 6d shows the relationship between decay time and impedance is also non- linear.

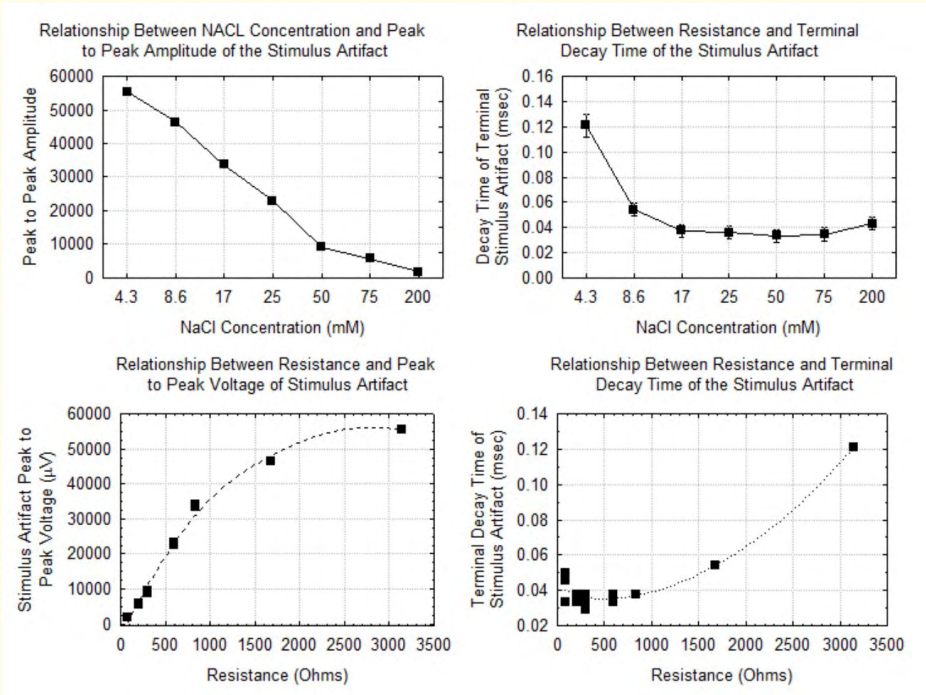

Figure 6: *With a 15 mA stimulation current (a) the change in peak-to-peak amplitude of the stimulus artifact with salt concentration, (b) the change in decay time with salt concentration, (c) change in peak-to-peak amplitude as a function of impedance, and (d) the effect of impedance on terminal decay time.*

Effect of Insulin

The addition of insulin up to 1000 nM (normal blood insulin concentrations are in the range of 0.1 nM) had no effect on impedance or viscosity and had only minor changes in stimulus artifact.

Effect of Oxygen

Figure 7 shows that deoxygenated solutions produced higher levels of stimulus artifact than oxygenated solutions without large changes in the shape of the waveform.

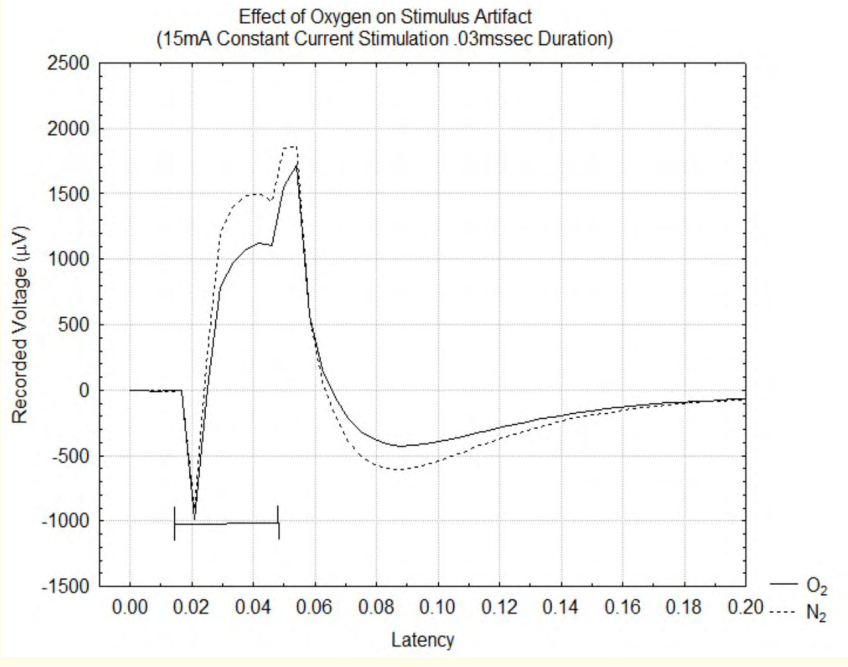

Figure 7: *The effect of oxygen on stimulus artifact. In the presence of oxygen, the stimulus artifact is reduced.*

Discussion

Stimulus artifact is a pervasive problem in neurophysiologic recordings. It is a complex phenomenon that is influenced by many factors, including the geometry of the stimulating and recording electrodes, electrode composition, characteristics of the electrical stimulus, and the properties of the solution or tissue in which the electrodes are embedded. The purpose of this paper was to show how some changes in a solution affect stimulus artifact in order to illustrate some important physical principles that can directly impact recorded neural signals.

The effect of increasing glucose in the solution was an increase in the peak-to-peak amplitude of stimulus artifact without changing the waveform shape. The explanation of this effect is relatively straightforward. Under constant current stimulation, Ohm's law requires that the voltage needed to produce a given stimulus current is proportional to the impedance of the solution (Appendix). Since stimulus artifact is proportional to the stimulus voltage, then under constant current stimulation, the amplitude of stimulus artifact will increase with the impedance of a solution. When a non-charged solute, such as glucose, is added to a solution, it increases the viscosity of the solution, effectively reducing the ability of charges to move through the solution and increasing the effective impedance of the solution (Appendix).

In these experiments, the effect of glucose on stimulus artifact only begins to become prominent at concentrations of 100 mM. Although this is beyond normal glucose concentrations (5 mM) or during severe hyperglycemia (55 mM), even a small 21 µV increase in stimulus artifact that might be expected with a 10 mM change in glucose concentration could become significant during a prolonged recording,

such as might occur during intraoperative neurophysiologic monitoring, especially if the neural signal being monitored is in the 1 - 10 μV range. This might be most important when recording a nerve action potential with closely spaced recording and stimulating electrodes.

The effect of salt concentration in the solution is very different from that of glucose.

One reason is a much larger range of impedance values can be explored by changing salt than by changing glucose. Although stimulus artifact decreases monotonically with the salt concentration and increases the impedance, the relationship is linear only in certain ranges of concentration and impedance. Figure 6c shows that stimulus artifact reaches a maximum value at large impedances, which could be due either to the constant current stimulator reaching its maximum voltage or due to chemical reactions occurring at the electrode-solution interface. One manifestation of the change in the stimulus artifact waveform with changing salt concentration is a much longer decay time for stimulus artifact in the setting of high impedance or low salt concentration. Since the duration of the stimulus pulse is only 0.03 ms and the duration of the stimulus artifact can be longer than 0.15 ms, there must be processes, such as discharging of the electrode capacitance or complex chemical reactions still occurring at the electrode surface during this time.

We did not show that low concentrations of macromolecules, like insulin, had a significant effect on stimulus artifact, but did show that the lack of oxygen had a significant effect. This may relate to reactions occurring between oxygen and iron at the electrode. This does suggest that under the right circumstances the amplitude of stimulus artifact can be used as an index of oxygen content (or lack thereof), which might have future clinical applications.

These data confirm the general principle that increasing viscosity, which increases resistance, will increase stimulus artifact under constant current stimulation. This general principle has wide ranging implications, not only in the clinical troubleshooting where an increase in resistance of an electrode can lead to exacerbation of the stimulus artifact, but also in predicting new effects. For example, because electrodes are placed in a tissue rather than in a pure saline environment, the electrical resistance of the tissue will affect the magnitude of stimulus artifact. Thus, stimulus artifact should provide some useful information about the tissue itself.

This opens the possibility that stimulus artifact is not just something to troubleshoot or eliminate, but is quite possibly an important neurophysiologic signal containing information about the medium in which the stimulus is produced. One potential future application might be in the assessment of cerebral ischemia during intraoperative neurophysiologic monitoring. During cerebral aneurysm surgery, recordings from the cortical surface can be made, and the stimulus artifact may change with ischemia since one of the first changes that occurs during a stroke is a decrease in the diffusion coefficient (and increased viscosity as noted in the Appendix) [9].

This idea is not completely novel, and the analysis of dynamic changes in electrode voltage and current have been used for many years. One of the techniques commonly used to analyze electrode properties is called cyclic voltammetry [10] in which the voltage on an electrode is repeatedly changed from a positive to a negative value and the current measured. As the electrical potential on the electrode passes that at which certain chemical reactions occur, there are sudden changes in the current moving through the electrode. This can be used to identify chemical reactions at the electrode and the presence of certain chemicals in the solution. It has been particularly useful as a non-invasive tool for measuring dopamine and other neurotransmitter concentrations in the brain [11]. Another common technique for analyzing electrodes involves the measurement of galvanostatic transients. This involves imposing a constant current through an electrode and recording the change in electrical potential needed to maintain that current [10].

As a final note, it is important to realize that the effects discussed in this paper would be markedly different if constant voltage stimulation instead of constant current stimulation. With constant voltage stimulation, the peak-to-peak voltage would be defined by the stimulator voltage and not by the impedance of the medium. However, there would be effects on the decay time after the end of the stimulus as described in this paper.

Appendix

From the macroscopic point of view, if σ is the conductivity of a solution, then the relationship between the local current density *J* and the electric field *E* is $J = \sigma E$. The key is relating the conductivity to the properties of the solution and ions. At the microscopic level, if a singly charged ion is placed in a solution consisting of many neutral molecules, then subjected to an electric field, it will collide frequentlwith neutral molecules and, on-average, will achieve a velocity given by $\vec{v} = \mu \vec{E}$, where $\vec{v}$ is the "drift velocity" and μ is the mobility of the ion. The current density is simply related to v by $J = cv$, where c is the net concentration of these ions.

This implies that the conductivity is related to the mobility by $\sigma = c\mu$ [12]. Relating a mechanical property of a solution, such as viscosity to the conductivity, at first glance seems somewhat unintuitive. In fact, it took Einstein in one of his three, so called annus mirabilis papers of 1905, to make this connection [13]. The Einstein relation is $D = \mu kT$ where D is the diffusion coefficient for the ion, k is the Boltzmann constant and T is the temperature. The Stokes-Einstein relation relates the diffusion coefficient to the viscosity η as

$$D = \frac{kT}{6\pi r\eta}$$ where r is the radius of the ion.

This can be used to relate the conductivity to the viscosity through the relation $\mu = \frac{1}{6\pi r\eta}$. The conductivity is then:

$$\sigma = \frac{c}{6\pi r\eta}$$

This demonstrates that the conductivity decreases and hence, the resistance increases, with increasing viscosity and with increasing ionic concentration, which is the fundamental relationship that describes the effects of salt concentration and glucose concentration in this paper.

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Volume 5 Issue 2 March 2017