In Vivo Rat Spinal Cord and Striated Muscle Monitoring Using the Current Interruption Method and Bioimpedance Measurements

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The therapeutic value of implantable neuroprosthetic devices would improve considerably if it were possible to monitor the electrical properties of the neural tissue, so that the stimuli to the nervous system can be optimally, efficiently and safely applied. The current interruption method (CIM) was previously described by the authors to obtain ex vivo electrical equivalent circuits (EECs) of rat liver and triceps surae (TS) tissues using a four-electrode arrangement, fractional calculus, and circuit theory. The aim of this paper was to investigate the use of CIM for in vivo real-time monitoring of rat spinal cord and striated muscle, as a prior step to addressing their potential uses in spinal cord injury or other conditions involving central nervous system (CNS) damage. Statistical analysis of the EEC parameter values was carried out to elucidate their physiological meaning and their possible uses in implantable neural prostheses.

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One of the more promising approaches to restoring or improving lost functions after central nervous system (CNS) damage, is the use of chronically implanted electrodes inside the CNS to modulate the neural circuit responses. For this purpose, the neuroprostheses use electrodes that allow muscles, nerves, spinal cord or brain to be stimulated.1 When an electrode is implanted chronically into the neural tissue, three distinct regions take part in the propagation of stimuli or in a biopotential measurement: (i) the electrode itself; (ii) the electrode/neural tissue interface; and (iii) the bulk neural tissue. Thus, an in depth knowledge of the electrical and electrochemical properties of each region is required for the design of optimal, efficacious and safe protocols of therapeutic value.2

The challenge regarding human neural interfaces consists in being able to record biopotentials and apply an appropriate stimulation protocol over the chronic timescales.3 Much effort has been devoted to electrode materials aimed at a better biointegration into the surrounding tissue and improvement in the electrical properties of the electrode/neural tissue interface.4–5 However, in the context of the implantable neuroprosthetics, no special attention has been paid to the electrical properties of the tissue itself and they are of great importance because, besides underlying its physiological state, they determine the propagation of the applied stimuli and consequently the therapeutic benefits. In general, the space distribution of the electrical tissue properties involves resistance (intra- and extra-cellular spaces) and capacitance (cell membrane separates the charge) leading to a frequency-dependent electrical impedance, other research has also considered the brain tissue component surrounding the electrode.5,6 Conversely, the frequency-dependent electrical properties of the spinal cord have been very poorly studied in the literature. Recently, impedance measurements of in vivo ovine spinal cord tissue involving the electrode/pial surface were carried out in the context of optimizing spinal cord stimulation protocols.7,8 However, a serious limitation is imposed by the sufficiently high conductivity of the cerebrospinal fluid (CSF) surrounding the neural tissue: the CSF shunts away most of the current that would have flowed through the tissue itself.9,10

Conventionally, bioimpedance measurements are obtained sequentially (a time-consuming process), that is, the electrical impedance at a specific frequency is obtained using a sinusoidal signal with the same frequency. For a drastic reduction of the measurement time, the current interruption method (CIM) was previously described by the authors to obtain ex vivo electrical equivalent circuits (EECs) of rat liver and triceps surae (TS) tissues using a four-electrode arrangement, fractional calculus, and circuit theory.2

The circuit shown in Figure 1a represents the EEC of a biological tissue. It comprises a resistance $R_I$ (extra-cellular medium) in parallel with the series combination of a resistance $R_M$ (intra-cellular medium), and a constant phase element (CPEM of parameters $Q_M$ and $\alpha$), which models the membrane’s capacitive properties. The integrity of the cell membrane is critical to maintaining the cell’s life. The parameters $R_I$ and $R_M$ vary slightly in normal conditions, but can be greatly modified in the presence of pathology (e.g., tissue inflammation due to trauma or infection will lead to extra-cellular edema and a consequent decrease in $R_I$).

Let a constant current $I$ (see Figure 1b) be applied to the EEC of Figure 1a for a sufficiently long time (much greater than the time constant $\tau$, with $\tau = (l/R_M+R_I)Q_M^{-1}$) and be abruptly interrupted at time $t = 0$. The resulting voltage $v(t)$ at the terminals of the EEC is sketched in Figure 1c.9 At instant $t = 0^-$ (just before the current is cut-off), $v(0^-) = R_M \times I$ which is the steady-state value. During the previous steady-state, CPEM acts as an open circuit (no current flows through the cell membrane) and all the current flows through $R_M$; the extra-cellular space carries all the current $I$ as shown in Figure 1d. At instant $t = 0^+$, immediately after interrupting $I$, the voltage is $v(0^+) = R_M \times I/(R_M+R_I)$. 

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The jump discontinuity, $v(0^+)-v(0^-)$, as shown in Figure 1c, is due to the instantaneous resistive response of the extra- and intra-cellular media. Just after that ($t = 0^+$), cell membranes discharge through the surrounding intra- and extra-cellular spaces (see Figure 1e), showing a fractional decay (Figure 1c) described by the one-parameter Mittag-Leffler function $E_{\alpha}(\frac{-t}{\tau})$. From an experimental transient-response $v(t)$, the parameter values of the EEC of Figure 1a are obtained as follows: (i) $R_0$ is found from the previous steady-state $v(0^-)$; (ii) $R_1$ is calculated from the value of $v(0^+)$; (iii) $\alpha$ and $\tau$ are determined by fitting the experimental decay of $v(t)$ to the expression $\frac{R_0}{R_1+R_0}E_{\alpha}(\frac{-t}{\tau})$ (see Figure 1c). Finally, $Q_M$ is obtained from the value of $\tau$.

The aim of this paper is to assess the CIM’s effectiveness for in vivo real-time monitoring of the electrical properties of rat spinal cord and striated muscle, as a prior step to addressing their potential uses in spinal cord injury or other CNS damage conditions. Note that the final result of an implanted device for motor recovery will depend on the physiological states of both neural and effector muscle tissues. An EEC is obtained for both types of tissues. For comparative purposes, conventional bioimpedance measurements were also obtained. We selected rat tissue samples because rodents are widely used as experimental animal models, and furthermore, both spinal cord and the striated muscles have been widely put to use as recipients of implantable neuroprostheses and/or regenerative devices to try to restore a number of human motor impairments.

**Experimental**

**Surgical procedure.**—The experimental protocols adhered to the recommendations of the European Commission and the Spanish regulations for the protection of the experimental animals (86/609/CEE, 32/2007, and 223/1988) and were approved by the Ethical Committee for Animal Research of the National Paraplegic Hospital of Toledo (Spain). Adult male Wistar rats ($n = 3, 16–24$ weeks old, $380–500$ g) were euthanized with an intraperitoneal lethal dose of pentobarbital (55 mg/kg) mixed with atropine (0.02–0.2 mg/kg) and xylazine (10 mg/kg), administering a supplemental 30% of these drugs 90 min after the initial dose. An unguent was applied to the eyes to prevent pain from corneal abrasion. Animals were kept at 36°C with the aid of a thermal pad.

The back and neck of the anesthetized animals were shaved and disinfected with povidone iodine. A dorsal midline incision was made in the skin and superficial muscles of the neck region, and blunt dissection was carefully performed to expose the cervical spine. The vertebrae C4 to C6, which containing the spinal cord segment C5 to C7, were identified by counting from the vertebrae C2 and T2. The spinous apophyses and dorsal laminae of C4, C5 and C6 were removed without applying pressure on the underlying spinal cord. Subsequently, a transverse incision was performed in the dura mater, so that the dorsal part of the metameras was exposed and prepared for the intraspinal placement of the four electrodes to acquire the electrical measurements. Once the spinal cord records were completed, a new incision was made in the dorsal skin over the left hindpaw to expose the TS muscle, in which the four electrodes were placed to repeat the electrical measurements with a similar scheme to that followed for the spinal cord (see below). After finishing data acquisition, rats were immediately euthanized with an intraperitoneal lethal doses of pentobarbital.

**Experimental protocol.**—Four-electrode setup consisted in four platinum needle electrodes (Grass Technologies, subdermal needle electrode, model F-E7: 10 mm long, 0.3 mm diameter) disposed in a straight line separated by an interelectrode distance of 4 mm, as shown in Figure 2a. Figures 2b and 2c illustrate the four-electrode arrangement. A sampling rate of 10 MHz was used. IS measurements were obtained by applying, between the two outer electrodes, a sinusoidal

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**Figure 1.** Current interruption method (CIM). (a) Electrical equivalent circuit (EEC) of the biological tissue, which involves a group of similar eukaryotic cells. Intra- and extra-cellular environments and cell membrane are shown in pink, blue, and green respectively. The black dots represent the cell nuclei. Waveform of the current injected into the tissue (b) and the transient-voltage response (c). Just before the electrical current is interrupted (at instant $t = 0^-$), the cell membranes are fully charged and the electrical current flows through the extra-cellular space (d). Just after the current is cut-off (at instant $t = 0^+$), cell membranes begin to discharge through the surrounding intra- and extra-cellular spaces (e).

The parameters of the EEC (Figure 1a) for the different tissues were determined by applying, between the two outer electrodes, a sinusoidal current. A sampling rate of 10 MHz was used. IS measurements were immediately performed using a linear sweep of the following parameters: DC to AC, $f = 1000$ Hz, sweep rate of $100$ Hz/s, and amplitudes of $10$ and $100$ mV peak-to-peak.
current of amplitude 50 μA with a frequency ranging from 1 MHz to 1 kHz (16 discrete frequencies logarithmically spaced). The AutoLab PGSTAT302N or PGSTAT204 Potentiostat/Galvanostat (Eco-Chemie) with the FRA32M module were used interchangeably to obtain the bioimpedance measurements. Each instrument was controlled by a computer and driven by NOVA software.

In each of the 3 rats, 5 recordings were acquired in the cervical spinal cord using the CIM with another final recording using the classical IS technique to corroborate the experimental data. Then, the same protocol was repeated in the left TS muscle. Only the EEC parameters obtained through the CIM were used for the statistical analysis.

Data analysis.—Statistical analysis was performed with the statistical package for Sciences Sigma Plot 11.0 (Inc, Chicago, IL). The EEC parameters were expressed as the mean value of the five trials ± the standard error of the mean (SEM). Comparisons between TS muscle and spinal cord were carried out using Student’s t-test or the Mann Whitney Rank Sum’s Test. The statistical significance levels were defined as follows: \( p < 0.05 \), \( p < 0.01 \), and \( p < 0.005 \).

Results and Discussion

Figures 3a and 3b show typical transient-voltage responses from the rat spinal cord and striated muscle tissues, respectively. The shape of the resulting voltage is similar to that predicted theoretically in Figure 1c. For the sake of clarity, only the samples taken every 0.2 or 0.4 μs are shown in Figures 3a and 3b, respectively. The previous steady state is shown during the 1 μs preceding the current interruption. Figures 3a and 3b also show the cell-membranes discharge during
the interval \( t = 0^+ \) to 12 or 30 \( \mu \)s, respectively. The parameter values listed in Table I were obtained by applying the procedure outlined previously. There is excellent agreement between the experimental and simulated data. For comparison purposes, we have also obtained the parameter values of the EEC of Figure 1a from IS measurements (NOVA software). Typical Nyquist plots (including experimental and simulated impedance data) are shown in Figures 3c (spinal cord) and 3d (TS muscle). Simulated impedance spectra have been obtained using the EEC of Figure 1a and the parameter values given in Table I. The parameter values of the EEC obtained using the CIM are remarkably close to those found with the IS technique. Note that the measurement acquisition time (\( \sim \) 1 ms) using the CIM is much shorter than that of the conventional bioimpedance measurements (\( \sim 30 \) s). The value of the current (50 \( \mu \)A) was sufficiently small to ensure linear operation of the biological tissue and sufficiently large to allow an acceptable signal-to-noise relation without causing damage to the tissue.

We have used a four-electrode arrangement in a straight line with an identical separation distance of 4 mm. The current injected between the two outer electrodes generates a potential distribution, which is frequency-dependent, in the tissue. The parameter values of the EEC of Figure 1a depend not only on the electrical properties of the tissue but also on the geometry of the measuring system.\(^2\) The question of how the parameter values of the EEC change with the interelectrode distance or the insertion depth is solved very effectively by using field theory,\(^2\) which is not the case here.

**Table I. Parameter values of the EEC shown in Figure 1a, obtained using the current interruption method (CIM) and bioimpedance measurements.**

| Biological tissue | Measurement method | \( R_I \) (\( \Omega \)) | \( R_E \) (\( \Omega \)) | \( Q_M \) (nF \( \mu \)s\(^{-1} \)) | \( \alpha \) | \( \tau \) (\( \mu \)s) |
|-------------------|--------------------|----------------------|----------------------|-----------------------------|-----------|----------------------|
| Spinal cord       | CIM                | 125.78               | 258.39               | 420.67                      | 0.71      | 4.57                 |
|                   | Bioimpedance       | 119.89               | 264.32               | 451.81                      | 0.70      | 4.25                 |
| Striated Muscle   | CIM                | 59.61                | 205.40               | 1156.94                     | 0.72      | 13.19                |
|                   | Bioimpedance       | 54.26                | 213.81               | 1259.87                     | 0.71      | 12.90                |

Figure 4a shows that the average \( R_I \) of the spinal cord almost doubled the average \( R_I \) of the TS muscle (131.037 \( \pm \) 2.108 \( \Omega \) versus 55.706 \( \pm \) 1.465 \( \Omega \) respectively; Mann-Whitney Rank Sum Test, \( p = 0.016 \)). The relationship between the average \( R_E \) of the spinal cord and that of the TS muscle is quite similar (Figure 4b), whereas the gap between them, though statistically significant, is smaller (253.889 \( \pm \) 2.049 \( \Omega \) versus 192.067 \( \pm \) 0.737 \( \Omega \) respectively; \( t \)-Test, \( p = 0.011 \)). The morphological heterogeneity of the spinal cord tissue compared to the striated muscle with axons of different sizes and with multiple areas of myelin sheaths interspersed with the nodes of Ranvier, which represent the alternation of insulator zones with others of good conductivity and different passive electrical properties,\(^2\) could partially explain this phenomenon. It is worth mentioning that in our experimental set-up, the edge of some of the recording electrodes placed in the spinal cord could be touching the bone of the vertebral body underneath. Moreover, the duramater over the spinal cord must be removed prior to electrode insertion, releasing the CSF and creating a highly conductive environment within the spinal cord\(^19\),\(^20\) which is not to present within the striated muscle during the recordings.

\( R_E \) was significantly higher than the \( R_I \) for both spinal cord (mean \( R_I 131.037 \pm 2.108 \Omega \); mean \( R_E 253.889 \pm 2.050 \Omega \); \( t \)-Test, \( p<0.001 \)) and TS muscle (mean \( R_I 55.706 \pm 1.465 \Omega \); mean \( R_E 192.067 \pm 0.737 \Omega \); Mann-Whitney Rank Sum Test, \( p = 0.029 \)) tissues. In a previous study with rat ex vivo TS muscle tissue,\(^6\) we found a noticeably higher \( R_E \) (231.3 \( \Omega \)). Although in both studies the target tissue was...
the same and the recordings were made with a similar set-up, the tissue temperature was not measured. Both ex vivo and in vivo recordings were made in a surgical area at room temperature (24°C), but the ex vivo TS muscle had been previously kept at 4°C after perfusion with paraformaldehyde 4% solution, whereas the in vivo TS muscle was maintained at 36°C with the aid of a thermal pad during data acquisition. In our opinion, the main difference between these two experimental designs is the muscle vascularization state. Whereas in ex vivo TS the perfusion process had fixed the blood vessels, in the anesthetized rats the TS vascularization was profuse and its blood vessels retained the ability to modify the muscle blood supply, so the higher the blood supply the lower the $R_E$. The average $\alpha$ spinal cord value was significantly lower than that of the TS muscle (0.651 ± 9.244 × 10^{-4} versus 0.665 ± 9.349 × 10^{-4} respectively; t-Test, $p = 0.001$) (see Figure 4c). As the $\alpha$ value quantifies the capacitive behavior of a particular tissue fragment considered globally ($\alpha = 1$, pure capacitor), this difference could also be due to the non-homogeneous distribution of the myelin sheaths along the axons, as opposed to the more homogeneous distribution of the lipid bilayer within the muscular cell membrane. This interpretation, although attractive, is speculative and should be carefully verified with other experimental set-ups of subcellular size and resolution.

Figure 4d represents the average spinal cord and TS $\tau \pm$ SEM values (Mann-Whitney Rank Sum Test, $p = 0.694$). Although we have not found statistically significant differences between the average $\tau$ values of the spinal cord and the TS muscle (Figure 4d), this apparent similarity ($p = 0.694$), which would indicate a similar electrical activation speed for both tissues, might not be accurate, since the calculation of the $\tau$ value takes into account the $\alpha$ value and therefore, its error could be transmitted to the global value of $\tau$. Thus, this “apparent similarity” should be treated with great caution.

Conclusions

The CIM is able to detect real-time differences in the EEC parameter values, modeling in vivo electrical properties of the spinal cord and TS muscle of the normal rat. The combination of high speed data acquisition that CIM permits, and the knowledge on the specific and differential electrical properties of these two target tissues will help to optimize the design of chronically implantable neuroprostheses and the electrical stimulation protocols.

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