Published in final edited form as:

J Surg Res. 2010 March ; 159(1): 557–564. doi:10.1016/j.jss.2008.10.014.

Regional Differences in Cerebral Edema after Traumatic Brain Injury Identified by Impedance Analysis

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Abstract

Objective—Cerebral edema is a common and potentially devastating sequel of traumatic brain injury. We developed and validated a system capable of tissue impedance analysis, which was found to correlate with cerebral edema.

Design and Methods—Constant sinusoidal current (50 μ A), at frequencies from 500 to 5000 Hz, was applied across a bipolar electrode unit superficially placed in a rat brain after traumatic brain injury. Rats were randomized to three groups: severe controlled cortical injury (CCI), mild CCI, or sham injury. At 60 hours post CCI, cerebral voltage and phase angle were measured at each frequency at the site of injury, at the penumbral region, at the ipsilateral frontal region, and in the contralateral hemisphere. Impedance measurements were also obtained *in vivo*. The electrical properties of varied injuries and specified locations were compared using a repeated measures analysis of variance (RMANOVA), were correlated with regional tissue water percentage using regression analyses, and were combined to generate polar coordinates.

Results—The measured voltage was significantly different at the site of injury ($p < 0.0001$), in the penumbra ($p = 0.002$), and in the contralateral hemisphere ($p = 0.005$) when severe, mild, and sham CCI rats were compared. Severely injured rats had statistically different voltage measurements when the various sites were compared ($p = 0.002$). The *ex vivo* measurements correlated with *in vivo* measurements. Further, the impedance measurements correlated with measured tissue water percentage at the site of injury ($R^2 = 0.69$; $p < 0.0001$). The creation of a polar coordinate graph, incorporating voltage and phase angle measurements, enabled the identification of impedance areas unique to normal, mild edema, and severe edema measurements in the rat brain.

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Authors contributions: MTH was involved in study conception, design, coordination, lab experimentation, statistical analysis, and drafting the manuscript. CTS was involved in study conception, design, and lab experimentation. RSR participated in background work, initial analysis, and study conception. KRA participated in the design, coordination, and lab experimentation. PKD participated in background work and study design. BG was involved in study design, coordination, and drafting the manuscript. CSC was involved in background work, study design, coordination, and drafting the manuscript.

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Conclusions—Electrical measurements and tissue water percentages quantified regional and severity differences in rat brain edema after CCI. Impedance was inversely proportional to the tissue water percentage. Thus, impedance measurement can be used to quantify severity of cerebral edema in real time at specific sites.

Keywords

traumatic brain injury; brain edema; trauma; electrical properties; impedance

Introduction

Cerebral edema is a pathological increase in the amount of brain tissue water. Development of cerebral edema is the result of a cascade of molecular and cellular, structural and functional, and local and systemic changes following an inciting event (1). The eventual clinical consequences of cerebral edema, irrespective of the inciting event, are often devastating, ranging from temporary minor disability to death. Depending on the type of insult, direct cellular injury and/or blood-brain barrier (BBB) breakdown can result in cytotoxic and/or vasogenic edema, respectively (1, 2). One insult that results in both of these major types of cerebral edema is traumatic brain injury (TBI). After an external traumatic force is applied to the brain, local damage and BBB breakdown lead to neurochemical mediator release and regional changes in cerebral edema.

Without tissue harvest, the ability to quantify cerebral edema remains imprecise. Imaging studies such as magnetic resonance imaging (MRI) and CT scanning remain the best options, in the clinical setting, for estimating severity of cerebral edema (3). Unfortunately, close monitoring of dynamic pathologic situations, such as cerebral edema, is currently impractical due to mobility, cost, and time restraints. An alternative strategy is to monitor intracranial pressure as a surrogate for cerebral edema. It is limited to measurement of global changes and usually requires confirmation via imaging to identify the site of developing pathology. Neither of these is a practical option in the experimental laboratory arena, due to cost, availability, and quality limitations, leaving wet to dry tissue weight ratios or density gradient measurements as the best current options (4, 5). Therefore, we pursued a technique to allow quantitative, real-time measurement of regional changes in cerebral tissue water without a tissue biopsy.

We hypothesized that the entry of water into traumatically injured brain tissue would be detectable as an alteration (likely decrease) in the impedance of the tissue to the flow of electrical current. In this study, we developed a system capable of brain tissue impedance analysis. We subsequently used the system to distinguish amongst varying degrees of edema associated with severity of traumatic brain injury and to identify regional differences in brain impedance. Further, we correlated electrical impedance measurements with brain tissue percent water content, establishing a novel and reliable method for determining real-time, site-specific severity of cerebral edema.

Materials and Methods

Impedance measurement

Eighteen Female Sprague Dawley rats (200–225 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN) for use in this study. The animals were housed on a 12-hour light/dark cycle with *ad libitum* access to food and water. All protocols involving the use of animals were in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee (protocol HSC-AWC-06-038). Rats were randomly divided into three groups of

six. The rats underwent *severe* (6 rats), *mild* (6 rats), or *sham* (6 rats) controlled cortical impact (as described below). The rats were euthanized 60 hours post injury, followed by immediate decapitation, and complete exposure of the brain. A consistent procedure was used to minimize contact with the brain, while maximizing time efficiency, to prevent further fluid shifts. A bipolar electrode unit connected to an impedance analyzer circuit was sequentially placed in brain regions relative to the location of the injury and electrical properties were measured in an automated fashion (as described below). Voltage and phase angle were measured for a logarithmic scale of varied frequencies at the following brain regions: (A) area of injury, (B) penumbral area, (C) ipsilateral frontal area, and (D) contralateral section parallel to the area of injury. Total measurement time for all sites was ~ 5 minutes.

Controlled cortical impact injury

A controlled cortical impact (CCI) device (eCCI Model 6.3; Custom Design) was used to administer unilateral brain injury as described previously. (6) Rats were anesthetized with 4% isoflurane and a 1:1 mixture of N₂O/O₂ and then mounted in a stereotaxic frame. The head was held in a horizontal plane, a midline incision used for exposure, and a 7-8 mm craniectomy was performed on the right cranial vault. The center of the craniectomy was placed at the midpoint between bregma and lambda, ~ 3 mm lateral to the midline, overlying the tempoparietal cortex. Animals received either a single impact of 3.3 mm depth of deformation with an impact velocity of 6 m/s and a dwell of 150 ms (*severe* injury) or a single impact of 1.5 mm depth, with an impact velocity of 5 m/s, and a dwell of 150 ms (*mild* injury) at an angle of 10° from the vertical plane using a 6 mm diameter impactor tip, making the impact orthogonal to the surface of the cortex. An audible baseline monitor was used to ensure that the location of the tip, relative to the surface of the brain, was consistent prior to each impact. The impact was delivered onto the parietal association cortex. *Sham* injury was performed by conducting all procedures, including craniectomy, except the impact injury. Although CCI injury, utilizing the same piston parameters we used, has been previously shown not to produce peri-procedural hypoxia, respirations were constantly monitored for all animals and serial ABG's checked during the procedure for an animal during this experiment to ensure that there was no evidence of peri-procedural hypoxia. The body temperature was maintained at 37°C by the use of a heating pad.

Placement of bipolar electrode unit for impedance analysis

The bipolar electrode unit (BEU) was purchased from Plastics ONE® (Cat # MS303/6-A; www.plastics1.com; Roanoke, VA, USA). The BEU consists of two platinum wire electrodes (0.33 mm in diameter; 1 mm apart) extending below a threaded cylindrical molded plastic pedestal. The electrodes are individually Teflon insulated. The threaded plastic pedestal accepts a captive collar connector cable.

After the animals were euthanized, decapitated, and the brain was completely exposed, the BEU was sequentially placed, with electrodes extending to a depth of 2 mm, in the same 4 brain regions relative to the location of the injury (described above). The BEU was connected to the impedance analyzer circuit. A programmable digital function generator (Model LXI #33220A; Agilent Technologies, Santa Clara, CA, USA) was programmed to provide a sinusoidal input voltage, which was converted to current by the impedance circuit, at frequencies from 500 Hz to 5000 Hz. A digital oscilloscope (Mixed Signal Oscilloscope #MSO6032A; Agilent Technologies, Santa Clara, CA, USA) was used to record output voltage and phase angle versus input in an automated fashion. The data were automatically recorded in a spreadsheet (Microsoft Excel Professional 2003) using an Agilent Visual Engineering Environment (VEE) Pro 8.0 interface. Complete data was recorded for each region. Total duration of measurement in each region was 30-60 seconds.

Impedance analysis in vivo

Given distinct differences in cerebral blood flow and electrical activity between *in vivo* and *ex vivo* brains, our desire to translate this work to an *in vivo*, real-time measurement system, and given known concerns over the development of cytotoxic edema after animal sacrifice, we performed similar impedance analyses on rats that were alive (with normal cerebral blood flow and brain activity). Sixty hours after a severe CCI was performed, as described above, rodents were anesthetized and their area of injury was re-exposed. The BEU was placed in the direct area of injury and voltage and phase angle were recorded as described above. Anesthesia was then discontinued and the animals were awakened. *In vivo* measurements were obtained on severely injured rats in the area of direct injury (n=4) and control rats (n=4).

Because the impedance of tissue to passage of a sinusoidal current is a complex quantity, both voltage (V) and phase angle (°) measurements, were combined into a single plot of impedance using polar coordinates. (7) In a polar coordinate system, each point on the 2D plane is determined by a distance (voltage) and an angle (phase angle). The graph was created with (V cos °) and (V sin °) on the X and Y axes, respectively. Polar data are presented as mean ± SEM in both axes.

Tissue water percentage measurement

Tissue water percentages were measured for the same brain regions. After impedance measurements were taken, the brain was immediately sectioned in the coronal plane. The 4 sections weighed were (A) area of injury, (B) penumbral area, (C) ipsilateral frontal area, and (D) contralateral area parallel to the location of the injury. Each section was weighed individually and then placed in a desiccator at 60° C for 72 hours. The tissues were then re-weighed. The tissues were weighed a third time, 24 hours later, to ensure a stable dry weight. The percent tissue water was then calculated using the following formula: (Wet Wt - Dry Wt)/Wet Wt × 100.

Statistical Analysis

After electrical and physical measurements, the data were entered into NCSS for statistical analysis. Voltage measurements over the frequencies 500-5000 were compared using a Repeated Measures Analysis of Variance (RMANOVA). The F ratio was used for significance testing. Voltage measurements at specific frequencies and percent brain water content were compared using an Analysis of Variance (ANOVA). For measurements where a statistically significant F ratio was identified, post-hoc comparisons were made using the Tukey-Kramer procedure. Linear regression was used to compare impedance (defined by the formula: $V \cos \theta$; see below) and the wet:dry tissue weight ratios. The t-test (versus a slope of zero) was used for significance testing. Results were considered significant at $p < 0.05$. All data points are presented as mean ± SEM.

Results

The measurement of voltage and phase angle, over frequencies in the range of 500-5,000 Hz, identified significant differences in the electrical properties of rat brains after varying levels of TBI. Measured voltages at the site of injury were significantly lower in the severely injured brains and in the mildly injured brains compared with the sham injured brains (Figure 1a) ($p < 0.0001$, RMANOVA). Measured voltages in the penumbral area and in the contralateral hemisphere were also significantly lower in the severely injured brains and in the mildly injured brains compared with the sham injured brains (Figure 1b and 1d) ($p = 0.002$ & $p = 0.005$, respectively). Measured voltages in the ipsilateral frontal area did not reveal significant differences when severe, mild, and sham injured rats were compared

(Figure 1c) ($p=0.287$). The same statistically significant differences are observed if phase angle is incorporated into the analysis (along with Voltage on the y-axis using the formula: $V \cos \theta$).

At a frequency of 5,000 Hz, sham, mild, and severe rats were significantly different at the site of injury ($p<0.0001$, ANOVA). The average voltage at the site of injury among the sham injury rats was 809.1 ± 41 mV. This was significantly higher than the voltage at the site of injury among the mild injury rats (613.5 ± 16 , $p<0.001$, Tukey-Kramer test) and the voltage at the site of injury among the severe injury rats (516.9 ± 12 , $p<0.001$). The same significance is seen irrespective of the frequency chosen to compare the voltages. A summary of the voltage measurements at a frequency of 5,000 Hz can be seen in Table I.

Comparison of different areas also revealed differences in the electrical properties of rat brain after traumatic brain injury. Among severe injury rats, the injury and penumbral areas were different from the ipsilateral frontal and contralateral areas. At a frequency of 5,000 Hz, voltages (mV) at the injury, penumbral, ipsilateral frontal, and contralateral sites were 516.9 ± 12 , 582.3 ± 39 , 662.3 ± 12 , and 650.7 ± 39 , respectively ($p=0.002$, ANOVA) (Table I). The voltage at the site of injury was significantly different from the ipsilateral frontal ($p<0.01$, Tukey-Kramer test) and contralateral ($p=0.01$) sites. Statistically significant differences were not seen when different areas were compared among the mild injury or sham rats.

In vivo measurements produced similar differences in tissue impedance. Measured voltages at the site of injury were significantly lower in the severely injured brains compared with the sham injured brains (Figure 2a). This corresponds to the behavior seen in the *ex vivo* model (Figure 2b). However, ANOVA results showed a significant difference in impedance for both sham and injury sites compared to their *in vivo* counterparts.

Differences in brain tissue water percentage by brain region and severity of injury were identified (Figure 3). At the area of injury and in the penumbral area, the tissue water percent after severe injury were significantly higher than after mild injury, and those after mild injury were significantly higher than after sham injury. These differences were not identified at other sites. Among the severe injury rats, the tissue water percent at the injury site ($80.04\% \pm 0.25$) was higher than in the penumbral area ($79.36\% \pm 0.15$) and significantly higher than in the contralateral hemisphere ($77.95\% \pm 0.10$, $p<0.001$, Tukey-Kramer test).

Differences in tissue water percentages correlated with differences observed in impedance measurements. At the site of injury, decreased impedance measurements (calculated by the formula $\text{Voltage} \times \cos \text{Phase Angle}$ ($V \cos \theta$)) correlated with increased tissue water percentage ($R^2 = -0.692$, $p<0.0001$, t-test against a slope of zero) (Figure 4). At both the injury and penumbral sites in the brain, impedance measurement was inversely proportional to tissue water percentage.

Creation of polar coordinates allowed the incorporation of voltage and phase angle measurements into a single plot of impedance (Figure 5). The distance of any point from the origin (the length of the vector, $|Z|$) represents the ability of the tissue to transmit an applied voltage (V) at the given frequency. The angle that Z makes with the X-axis, the phase angle (θ), represents the time delay associated with traveling through the tissue. This polar coordinate graph enables the identification of impedances unique to normal, mild edema, and severe edema measurements in the rat brain. Thus, measurements from a brain of unknown edema could be plotted on this graph, revealing the severity of brain edema.

Discussion

Our data revealed that brain tissue impedance measurement is a reliable method of determining *ex vivo* and *in vivo* changes in cerebral edema after a controlled cortical impact injury in a rat model of traumatic brain injury. Voltage and phase angle electrical measurements clearly distinguished severity of CCI injury, when sham, moderate, and severe injuries were compared. The impedance measurement correlated with commonly used regional brain tissue water content measurements. A two-dimensional polar coordinate plot was generated, allowing the identification of edema severity based on impedance measurements in the rat brain.

Traumatic brain edema has been classified as focal, perifocal, or diffuse based on location of increased tissue water relative to traumatic impact (1). The rat model of controlled cortical impact injury has been shown to induce focal and perifocal edema (1, 8). Perifocal edema is located in an area also known as the *traumatic penumbra*, an area of injured but still viable brain tissue (9). Studies of the cat brain after impact-acceleration injury to the skull have identified regional differences in edema based on rostral-caudal location and severity of injury (10-12). Tornheim and colleagues used density gradient measurements to examine the topography of edema in the cerebral cortex and the white matter, identifying both vasogenic and cytotoxic edema in a “halo” area surrounding the site of local hemorrhage. They also identified a proportional relationship between edema and severity of injury. Sites located caudal to the injury and contralateral to the injury were found to have insignificant changes in tissue edema. Others have corroborated the finding that edema is localized the traumatized hemisphere (13). Our work confirms the significant focal and perifocal increases in edema after injury but also identifies increases in contralateral hemisphere edema. This is likely due to the different time points of study, as we conducted our experiments at a later time point than most of the studies referenced, but may also be attributable to a higher sensitivity of our technique.

General understanding of the timing and evolution of brain edema is the result of years of experimental study and scrutiny. Previous study has identified significant increases in brain tissue water as early as 15 minutes after injury (13). Many early studies of brain trauma focused on the acute development of edema (i.e. less than 24 hours) (10-12, 14). Previous experimental study of rats after CCI injury has shown that brain edema is maximal between 24 and 72 hours (15-17), and resolves by 7-10 days (13, 16). Our studies were conducted at 60 hours after CCI, with the goal of measuring maximal levels of brain tissue edema. Although no precise time-point exists for the measurement of maximal edema, nearly all studies agree that resolution of edema does not begin to occur until 4-5 days after insult.

In addition to the timing of measurement, another variable to consider when taking impedance measurements is the appropriate range of frequencies. We focused our analysis on frequencies in the 500-5000 Hz range, although measurements were obtained between 250 and 100000 Hz. Increases in tissue water secondary to trauma coincide with a host of other physiologic changes including electrolyte shifts, protein movement, and cell permeability alterations. These pathophysiologic changes are best characterized electrically in this model in the frequency range 500-5000 Hz. In addition, frequencies >10,000 or <500 reach the limits of this model and experimental setup for detecting differences.

The *in vivo* data are an important step toward translating these correlations to continuous, real-time measurement. In order to compare measurements of cerebral edema by both impedance and tissue weight ratio analyses, euthanization of the animal at the time of measurement was necessary. However, measurement in the *ex vivo* brain could differ from an *in vivo* brain due to cessation of blood flow, cessation of normal electrical activity, and

the development of cytotoxic edema. Although measurements were only obtained in one area, our *in vivo* data show that similar changes in impedance were identified. Continual time course experiments raise several additional challenges, including the development of scar tissue and infectious complications, which are under current investigation and comprise the next phase of this work.

Intense investigation is aimed at developing ways to identify and quantify cerebral edema, along with other pathophysiologic consequences of traumatic brain injury. Continual refinements in the area of brain imaging have led to significant improvement in image quality. MRI and CT scanning are two common modalities used in the clinical (3) and, more and more, in the experimental arenas (18-20). When MRI and CT scanning reveal normal brain structure, as is often the case with mild TBI, functional MRI (fMRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) are emerging novel imaging modalities in the clinical setting (21). Given the dynamic nature of cerebral edema, imaging is limited, only revealing one moment in a continuum. In addition, cost, time, and transport considerations inhibit the ability of current imaging to effectively monitor and/or quantify cerebral edema, in both clinical and experimental arenas. Intracranial pressure monitoring is an invasive means of tracking change in ICP, often the result of cerebral edema (22). This type of monitoring has the advantage of real-time evaluation, however, lacks the ability to identify the location of the pathology and requires confirmation via MRI or CT. Ueda and coauthors used transcranial measurement of diffuse light reflectance to noninvasively monitor brain edema in rats (23). By developing a minimally invasive means of electrical impedance measurement in realtime, at specific brain locations, our goal is to advance current knowledge of brain edema and improve our ability to monitor brain edema, cytotoxic and vasogenic, in experimental and, eventually, clinical settings.

The study of cerebral impedance began more than 50 years ago when van Harreveld and Ochs reported changes in cerebral impedance after circulatory arrest (24). Since that time, significant advances in electrical impedance measurement, specifically regarding the development of cerebral edema and hypoxic/ischemic injury, have been reported.(25-27) Our laboratory first used impedance analysis technology to measure intestinal edema (28). Impedance measurements have been shown to reliably detect carcinoma, inflammation, and edema formation in finite element models (29). Shochat et al used internal thoracic impedance monitors to predict cardiogenic pulmonary edema in patients with cardiac disease (30). Liu and colleagues explored non-invasive monitoring of cerebral electrical impedance in patients with intracerebral hemorrhage or cerebral infarction (31). The sensitivity of these measurements was high when the volumes were large or located in the basal ganglia, otherwise the sensitivity was low. Therefore, impedance measurement may prove to be a minimally or non-invasive way to evaluate a wide array of tissues and tissue pathologies.

In an intracranial setting, limiting the amount of injected current and maintaining high carrier frequencies eliminates any electrically derived neural effect attributable to the impedance measurement. Siemionow et al performed impedance measurements during human pallidotomy (32). In their study, one electrode was stereotactically directed towards the globus pallidus while the reference electrode was placed on the leg. Our study's bipolar electrode configuration limits the current pathway to a 2 mm distance. Applying electrodes directly into brain tissue has the potential for physical damage, but this can be minimized by having proper electrode geometry and gentle insertion techniques.

There are several limitations to this work. In order to correlate the impedance measurements with the most accurate and commonly used method of cerebral edema quantification (tissue

water percentage), the measurements had to be taken as rapidly as possible, with subsequent sectioning, weighing, and placement into the oven for drying. While our automated system maximizes the efficiency of this process, there remains a 3-5 minute span where decapitation, impedance measurement, sectioning, and initial weighing occurred. In this time, cytotoxic edema is likely developing, altering the impedance measurements. Second, the use of a bipolar electrode system (as opposed to four electrodes) could lead to a polarization of the electrodes which can occur when current is injected through the same electrodes that measure voltage. We chose the bipolar system to limit trauma to the implantation area, accepting the drift associated with electrode polarization that can reduce overall precision and accuracy. Finally, although it is known that cerebral edema after TBI is the result of both cytotoxic and vasogenic edema, the relationship between and relative contributions of cytotoxic and vasogenic edema remains unclear, and our impedance measurements do not differentiate between the two.

Our work builds on previous cerebral impedance work in the following ways: 1) we leveraged current technology to take measurements more accurately and throughout a wider frequency range than previous studies; 2) all current generation, measurements, and data logging were automated and able to be done very rapidly, ensuring that the physiology does not change over the course of the measurement; 3) we confirmed our measurements in an *in vivo* system, a prelude to continued work using repeated measurements *in vivo*; 4) we correlated our electrical measurements in specific regions with tissue water percentage in the same regions, and 5) given our voltage and phase shift measurements, we generated polar coordinate plots and identified unique, 2 dimensional regions therein which may help predict severity of injury.

We have developed a method to correlate electrical changes in brain tissue with percent brain water content at specific cerebral locations. Such electrical measurement could replace wet:dry tissue weight ratio determination and density gradient evaluation, minimizing animal numbers needed for time course studies. This technology could also be used to identify severity of injury if unknown, monitor *in vivo* temporal evolution of cerebral edema, or evaluate experimental therapy geared toward minimizing edema.

Acknowledgments

Supported by NIH grant: T32 GM008792-06

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A

B

Figure 1. Comparison of voltage measurements by injury severity at certain locations
Sixty hours after severe, mild, or sham CCI, the brain was exposed and electrical measurements were taken at 4 locations: **A)** Area of direct injury – measurements revealed a significantly lower voltage in the severe and mild injury groups compared to the sham injury group ($p < 0.0001$, RMANOVA). **B)** Penumbra area – measurements revealed a significantly lower voltage in the severe and mild injury groups compared to the sham injury group ($p = 0.002$, RMANOVA). **C)** Ipsilateral frontal area – measurements revealed no difference in voltage among the groups. **D)** Contralateral area – measurements revealed a significantly higher voltage in the sham group compared to the mild or severe injury groups ($p = 0.005$, RMANOVA). (The results are similar and the same statistically significant differences are noted if phase angle (ϕ) is incorporated into the y-axis using the formula $V \cos \phi$.)

Figure 2. Comparison of voltage measurements in the area of direct injury in vivo

A) Sixty hours after severe (n=4) or sham (n=4) CCI, the brain was exposed, while the animal was under anesthesia and monitored for continued normal cardiac function, and electrical measurements were taken. The measurements revealed a significantly lower voltage in the severe injury group compared to the sham injury group ($p=0.01$, t test at 5,000 Hz). **B)** Comparison of severe CCI measurements between *in vivo* and *ex vivo* groups. *In vivo* results for severe injury yielded statistically significant differences in voltage range values (0.3-1.2) compared to the *ex vivo* measurements (0.4-1.4) ($p<0.005$, t-test at 5,000 Hz). This testing validated our measurements in an *in vivo* system, confirming that the magnitude of the change is similar and that differences between sham and injured brains can be identified.

Figure 3. Tissue water percentage comparison by site of measurement and severity of injury
Brain tissue water percentage increased as severity of injury increased. In the severe injury rats, tissue water percent was decreased in the contralateral area compared to the direct injury area.

Figure 4. Correlation between impedance measurement and tissue water percentage
Voltage and phase measurements, as represented by the formula Voltage cos Theta ($V \cos \theta$) were inversely proportional to tissue water percentage at the site of direct injury.

Figure 5. Polar coordinate graph of impedance

which is a combination of voltage (V) and phase angle (θ). Because impedance is a complex quantity, both of these coordinates represent independent variables. Different areas of the graph represent different impedances; these data suggest that the degree of injury can be determined simply by which region of this chart the measured impedance falls within. Frequency increases from top to bottom within a given group. Error bars are $\pm$ SEM.

Table 1
Comparison of electrical properties at different locations in rat brain after CCI

The voltage measurements shown were taken at a frequency of 5,000 Hz (and are representative of the relationship of the measurements across all frequencies). ANOVA values compare measurements of the row or column at a frequency of 5,000 Hz. RMANOVA values compare measurements of the column across frequencies from 500–5,000 Hz.

CCI Injury Severity	Voltage at measurement site* (mV ± SEM)				ANOVA* (p value)
	Injury	Penumbra	Ipsilateral	Contralateral	
Sham	809.1 ± 41	809.1 ± 41	713.3 ± 28	809.1 ± 41	
Mild	613.5 ± 16 [‡]	638.0 ± 13 [‡]	647.2 ± 32	645.5 ± 21 [‡]	0.673
Severe	516.9 ± 12 ^{‡‡}	582.3 ± 39 [‡]	662.3 ± 12	650.7 ± 29 [‡]	0.002
ANOVA* (p value)	<0.001	<0.001	0.188	0.003	
RMANOVA** (p value)	<0.001	0.002	0.287	0.005	

* Frequency: 5,000 Hz

** Across frequency range: 500–5,000 Hz

[‡] Tukey-Kramer Test vs. Sham, p<0.05

^{‡‡} Tukey-Kramer Test vs. Mild, p<0.05