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Sustained Focal Cortical Compression Reduces Electrically-Induced Seizure Threshold

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Abstract

Brain injury can often result in the subsequent appearance of seizures, suggesting an alteration in neural excitability associated with the balance between neuronal excitation and inhibition. The process by which this occurs has yet to be fully elucidated. The specific nature of the changes in excitation and inhibition is still unclear, as is the process by which the seizures appear following injury. In this study, we investigated the effects of focal cortical compression on electrically-induced localized seizure threshold in rats. Male Long Evans rats were implanted with stimulating screw electrodes in their motor cortices above the regions controlling forelimb movement. Initial seizure threshold was determined in the animals using a ramped electrical stimulation procedure prior to any compression. Following initial threshold determination, animals underwent sustained cortical compression and then following a 24 hour recovery period had their seizure thresholds tested again with electrical stimulation. Reliability of threshold measurements was confirmed through repeated measurements of seizure threshold. Localized seizure threshold was significantly lowered following sustained cortical compression as compared to control cases. Taken together, the results here suggest a change in global brain excitability following localized, focal compression.

Keywords

traumatic brain injury; spreading depression; excitation and inhibition

Focal cortical compression can be a significant problem following or contributing to several types of brain injury pathophysiologies, such as traumatic brain injury, brain edema, focal cerebral ischemia, or brain tumors (Watanabe et al., 2001, Chen et al., 2004, Salci et al., 2006, Yang et al., 2006). Mechanical deformation of neurons, even when the perturbation seems minor, can result in delayed neuronal injury or death (Arundine et al., 2003) and the

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effects of increased cortical pressure can subsequently manifest as cerebral infarction (Marino et al., 2006), increased extracellular potassium (Reinert et al., 2000), and as alterations in neuronal firing rates (Maeda and Miyazaki, 1998). Functionally, these mechanical and cellular alterations can result in global changes in excitation and inhibition on the neuronal network level (Lee et al., 1997, Wang, 2003, Arundine and Tymianski, 2004, Glass et al., 2004, Adamo et al., 2005). Seizures often occur in patients who have experienced traumatic brain injury, with one study reporting an incidence as high as 22% following moderate to severe head injury (Vespa et al., 1999), and their appearance is considered a sign of the resulting imbalance between excitation and inhibition from the trauma (Loscher, 1985, D'Ambrosio et al., 2004, Luszczyk et al., 2007).

Considering these changes in excitation and inhibition and the frequency of spontaneous seizure occurrence after head injury (Claassen et al., 2003), it is possible that induced seizure thresholds could also change following brain trauma or other mechanical injury, thereby providing a controlled way in which to quantify the changes in excitability that result. Since seizures can involve large portions of the brain even if they have a focal origin, the seizure threshold can be considered a gross measure of overall brain excitation. Establishing that induced seizure threshold is altered following brain injury would provide further evidence that the balance between excitation and inhibition is altered in pathophysiological settings and that these changes can be observed from a functional perspective. To our knowledge, there are no studies that directly investigate the alterations in seizure threshold following brain trauma or focal cortical compression with the use of electrically induced localized seizures.

In this study, we investigated the effects of chronic focal cortical compression on the effects of localized forelimb seizure threshold elicited via direct cortical stimulation in rats. Previous studies have suggested that using ramped cortical electrical stimulation is an efficient and effective method for determining the seizure threshold (Voskuyl et al., 1989, Krupp and Loscher, 1998). Ramped electrical stimulation is advantageous relative to pharmacologic methods since it provides a relatively quantitative method for assessing seizure onset and propagation and also allows the seizure threshold to be repeatedly tested in the same animal (Krupp and Loscher, 1998). By creating a focal cortical compression model *in vivo* in rats, we attempt to evaluate changes in brain excitability that may be similar to those experienced following traumatic brain injury. Evaluation of these changes in a relatively acute period following injury could provide insight into electrophysiological studies as well as contribute some potential avenues for more effective clinical intervention.

Experimental Methods

To investigate the effects of cortical compression on seizure threshold, we used 21 male Long Evans rats (Charles River, 250–350 g, n=5 control animals, n=16 compression animals), monitored over a period of 3 days. All procedures were approved by the Animal Care and Use Committee at Harvard University and in accordance with National Institutes of Health guidelines. On the first day, rats were anesthetized with 1.5%–2% vaporized isoflurane before being transferred to a stereotaxic frame (Kopf Instruments, Tujunga, CA) for surgery. Body temperature was maintained at 37.5 ± 0.5 °C with a feedback-controlled heating pad throughout the procedure. A craniotomy was then performed over the animal's left parietal bone, resulting in the removal of an approximately 2.5 mm × 2.5 mm region of skull extending approximately from 0.5 mm – 3.0 mm caudal and 0.5 mm – 3.0 mm lateral to the bregma. Two wired screw electrodes were then implanted in the skull at 1 mm anterior to the bregma and ± 3.5 mm lateral positions of the skull (over motor cortex) as shown in figure 1A. The screw electrodes were secured at a depth of approximately 0.5 mm from the surface of the skull so that they touched the cortical surface, but did not generate significant cortical deformation. A sterilized piece of silicon was then placed over the craniotomy to protect the exposed brain. The skin flaps were

sutured and disinfected. The rats then received injections of Flunixin (1 mg/kg) for analgesia immediately following surgery and were returned to their cages for a recovery period of 24 hours.

Following this initial recovery period, the rats were removed from their cages and placed in a transparent observation chamber. The screw electrode wires were connected to a linear stimulus isolator (World Precision Instruments, Model A395). The rats then received ramped bipolar current stimulation directly to their cortex with a stimulation protocol (see figure 1B) modified from (Krupp and Loscher, 1998) and (Voskuyl et al., 1989). These animals were videotaped as they received ramped 50 Hz bipolar current pulses (each bipolar pulse is larger than the previous by 1.96 μ A; maximum stimulus amplitude did not exceed 4 mA) passed through the screw electrodes until localized seizure, the criterion for which was forelimb clonus, was observed. Once forelimb seizure was observed, stimulation was immediately halted and the seizure threshold was recorded. The initial experiments were performed with partial blinding and utilizing two experimenters. One experimenter was an observer and was solely responsible for stating when localized seizure began, while the other experimenter controlled the onset of stimulation without the observer's knowledge, timed the session and halted the stimulation when the observer stated observation of seizure initiation. Forelimb clonus and seizure threshold during a given session were almost always very consistent and reproducible with variations in seizure thresholds typically being less than 0.2 mA, which is a minor deviation, as can be seen in figure 2B. Only one forelimb would exhibit seizure and although it could not be predicted beforehand whether it would be the animal's right or left forelimb that would exhibit seizure activity, the same forelimb would always exhibit clonus during stimulation trials in a given rat.

During a given stimulation session, the ramp stimulation was repeated 5 times with 3 minutes of rest in between stimulus trains in order to evaluate consistency and accuracy for seizure threshold in the session. The stimulating electrodes were disconnected in between stimulation trials to stop current flow and minimize buildup of electrical charge in the brain. The recordings were later reviewed to verify seizure occurrence and current amplitude at which seizures occurred. Control animals were returned to their cages for another 24 hours, after which the stimulation test was repeated prior to sacrificing the animals with Euthasol solution immediately following this stimulation trial (approximately 48 hours following the initial surgery).

Animals receiving cortical compression were not returned to their cages and were instead re-anesthetized and placed back into the stereotaxic frame under isoflurane anesthesia. In order to create focal compression on the cortex, a disinfected Parafilm-coated steel bead (2 mm diameter) was used to contact the surface of the brain. The Parafilm coating was used in order to prevent or minimize chemical interactions between the metal and the brain tissue and to prevent any undesired electrical conduction. The bead was first placed onto the craniotomy site, and then a disinfected Parafilm-coated stainless steel plate sized to extend over the craniotomy was placed over the craniotomy until it was flush with the skull surface, causing the ball bearing to compress a focal portion of the brain. The plate was secured to the skull with adhesive glue and dental acrylic and after the acrylic had dried and solidified, the scalp flaps were tightly closed and sutured again. Following 24 hours of recovery, cortical compression animals received another seizure threshold evaluation session identical to the process that the control animals underwent and were subsequently sacrificed immediately following their final stimulation trial. All surgeries were performed by the same surgeon to ensure consistency in the surgical procedure.

Although electrical stimulation in this model occurred across the motor cortex, cortical compression was generated slightly posterior to the area of stimulation. Brain injury can cause

cortical spreading depression (Nilsson et al., 1996), which potentially affects brain excitability in exposed regions and possibly plays a role in seizure threshold alterations (Bough et al., 2003). Thus, injury in this model was created in a region near the stimulation site to observe the effects of spreading injury.

Following sacrifice of the animals, brains were perfused and standard procedures were used to obtain DAPI and TUNEL staining to assess cell death resulting from the cortical compression or the stimulating electrodes (Matsushita et al., 2000, Nguyen et al., 2007).

Results

Results were compiled and analyzed using STATA (StatCorp, College Station, TX). An unpaired t-test was performed on the control group of animals and on the cortically compressed animals. The two groups were significantly different, as assessed using an unpaired t-test with unequal variances ($p=0.010$) with the animals undergoing compression averaging a decrease in seizure threshold of -0.40 ± 0.22 mA or $-14\% \pm 4.0\%$ ($\pm 95\%$ CI) versus an average change of -0.051 ± 0.17 mA or $-1.4\% \pm 2.4\%$ ($\pm 95\%$ CI) in control animals. The results are summarized in figure 2, showing the sample average percent change in threshold (fig. 2A) and a scatter plot of the day 1 versus day 2 threshold for both control and compression groups (fig. 2B).

Histological assessment of the brains indicated that scattered instances of cell death were detectable immediately under the region of compression and immediately adjacent to the stimulating electrodes, as shown in figure 3. However, no signs of injury in cortex or any other part of the brains were observed, indicating that the functional perturbations in electrical thresholds were not due to widespread brain damage or cell death in this model.

Discussion

It is important to note that in previous studies using this model of seizure induction, animals were stimulated for periods of 1–2 weeks prior to determining a stable seizure threshold. Although in some experimental conditions electrically induced seizures may need a “settling period” before reaching a true steady state value (Voskuyl et al., 1989, Krupp and Loscher, 1998), we observed that the threshold values remained fairly constant between trials, at least over the relatively short time period of one day during which we were generating forelimb seizures. Figure 2B indicates that a small number of animals undergoing cortical compression did not demonstrate significant decreases in seizure threshold. This could be the result of different susceptibilities to injury between different animals. Despite these minor variations in individual cases, across the larger sample our results indicate that the effects of focal cortical compression consistently cause significant decreases in seizure threshold over a 24-hour period. These reductions were more than would be expected from typical variation, as evidenced by the difference in changes between our control and injury groups.

It has been established that seizures can result from disruption of the balance between excitation and inhibition. To our knowledge, the results of the present study are the first to demonstrate that a focal chronic cortical compression can alter excitation and inhibition, resulting in changes in localized seizure threshold. Currently in clinical settings, intracranial hematomas are emergently evacuated via surgery if the hematomas are greater than 10 mm in thickness (Servadei et al., 2007). If this condition is not met, then generally a more conservative approach is taken. The results of this study suggest that even focal compressions, when sustained, can result in changes in overall brain excitation and inhibition. These changes can result in adverse outcomes, such as increased incidence of seizures, but may also play a role in the brain’s ability to recover from injury, especially considering that excitotoxicity is often cited as a major cause

of neuronal cell death. The decreased seizure threshold that this study demonstrated suggests that cortical compression changes the excitatory-inhibitory balance, either through an increase in excitation, decrease in inhibition, or both.

The limited cell death implies that this imbalance is not the result of preferential elimination of inhibitory or excitatory neurons, but may possibly be due to functional changes in the behavior of neural networks. Neurons have been shown to be extremely sensitive to even “sublethal” mechanical manipulation and neuronal deformation alone appears to activate a variety of signaling pathways in an *in vitro* setting (Arundine et al., 2003, Arundine et al., 2004). Thus, these results raise the possibility that the observed imbalance between excitation and inhibition following focal cortical compression is the consequence of compressed neurons activating normally dormant or deregulating signaling pathways, which changes their fundamental neuronal firing patterns and properties. Abnormal activity from a concentrated group of neurons could then potentially alter activity on a large scale due to the effects of their activity on neural networks and this could also possibly explain the apparent global changes in excitation and inhibition from focal compression.

Seizures are typically indicative of a poorer outcome following head injury. Thus, the presence of a focal cortical compression and the subsequent disruption in the balance between excitation and inhibition following brain trauma likely leads to a less favorable outcome. Alleviating abnormal levels of excitation and inhibition could prove to be beneficial to final clinical outcome. This study provides a gross examination of functional changes resulting from cortical compression. Further work utilizing electrophysiology or other techniques that can more precisely evaluate the functional changes that occur in the injury setting could provide greater insight into the specific alterations in excitation and inhibition that often result after brain injury.

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Abbreviations

CI

Confidence Interval

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Figure 1. Experimental setup for focal compression and current injection

(A) The location of the screw electrodes and the craniotomy/compression site relative to cranial sutures from a top view of the animal's skull. (B) A sample waveform for the ramp stimulation, illustrating how seizure threshold was defined. When seizure occurred, the timestamp was noted and the electrical current amplitude corresponding to that time defined the seizure threshold for that trial. Note that this waveform is for illustrative purposes only, and that the actual ramp stimulation protocol increased the current amplitude in a much more gradual fashion (see text).

Figure 2. Seizure threshold decreased following cortical compression

The differences in seizure thresholds were obtained by evaluating the difference between the seizure threshold on the second day relative to the first (day 2 threshold – day 1 threshold) in mA. **(A)** A comparison of the percent change in threshold on the second day of stimulation relative to the first day of stimulation in both the animals receiving no compression (control, filled) and the animals that received focal cortical compression (compression, open). Error bars represent the standard error of the mean (SEM) across animals. **(B)** The scatter plot shows the thresholds of the animals on the first day relative to the second (control – filled symbols; compression – open symbols). The center solid line of unity slope represents an idealized situation in which the thresholds would not change between the first and second day. The outer

lines represent the 95% confidence intervals of the control population. Error bars for each point represent the standard error of the mean (SEM) for the threshold across repeated threshold tests for a given animal.

Figure 3. TUNEL and DAPI staining revealed minimal cell death

(A) Histological coronal sections were taken from 3 separate regions of the brain. Section 1 was taken from underneath the stimulating electrodes. Section 2 was taken from the injury site. Section 3 was taken from an area caudal to the craniotomy site. (B) DAPI and TUNEL staining procedures indicate presence of cell nuclei and cell death, respectively. Staining show sparse cell death immediately adjacent to the stimulating electrodes and underneath the compression site, but overall, there is very little sign of injury. Scale bar = 100 μ m.