**Abstract:**

Traumatic brain injury (TBI) is the leading cause of death for people under the age of 45 (Gabrielian et al, 2011). One of the most troubling problems for physicians in treating TBI is increased intracranial pressure (ICP). ICP is the pressure the brain and the cerebrospinal fluid exert on the skull. In a study consisting of TBI patients, 54% of them had increased ICP (Rangel-Castillo, 2008). Experimental studies show variable results, with some injury models exhibiting a significant increase in ICP whereas others do not. This controversy calls for more research to clarify under what conditions experimental TBI mimics the ICP changes that occur clinically. One of the difficulties is that the methods in which ICP is measured are variable, or are not detailed enough to compare from study to study. For example, the methods do not detail, during open head injury, whether the bone flap is replaced (craniotomy) or not (craniectomy). For this research study, the differences between the two methods, craniotomy versus craniectomy, will be studied to see if there is a significant difference between the two methods. The data showed no significant difference for either trial, but the results show that the data is more consistent with the use of the craniotomy.

**Literature Review:**

After clinical TBI, one of the main physiological changes measured to rate the extent of the trauma is ICP. A long period of ICP increases contributes significantly to mortality and morbidity (Steiner and Andrews, 2006; Eide et al., 2007). TBI clinical management is largely focused on controlling physiological disruptions, such as hypo/hypertension and ICP that are the result of secondary injury. A primary goal in
clinical TBI management is minimizing the period of time in which ICP is increased, which is a risk factor for morbidities and mortality. Some common contributing factors associated with increased ICP levels are trauma, bleeding, tumors, radiation, and infections, which can lead to hampered blood circulation, metabolic disturbances, mechanical distortion, and eventually brain herniation and death (Baringer, 2000; Johnson, 1998; Love and Wiley, 2002; Raschilas et al., 2002; Steiner et al., 2005; Tyler 2004; Whitley, 2006). The clinical importance of this cannot be overstated; most secondary injury processes contribute (directly or indirectly) to increased ICP and the secondary injury unfolds minutes, hours, and days following TBI, a period often under clinical monitoring. Currently, increased ICP is treated with two main techniques: first is directly by draining the cerebrospinal fluid or giving mannitol, the second is indirectly by inducing hyperventilation, giving barbiturate therapy, or causing hypothermia. These may or may not cause permanent relief of increased ICP (Steiner et al., 2005). For this reason, it is not only important to measure or monitor ICP but also understand the mechanisms of elevated ICP so clinicians can treat TBI more effectively.

**Background:**

ICP is the pressure inside the skull relating to the tissue of the brain and the cerebrospinal fluid. The ICP under normal conditions is controlled by cerebral autoregulation, indirectly. Cerebral auto regulation is defined as the brain’s intrinsic ability to maintain adequate blood flow despite alterations in cerebral perfusion pressure (CPP) (Lewis, 2008). CPP is the difference between the mean arterial pressure (MAP) and the ICP. This pressure gradient is what controls cerebral blood flow. For injured
patients, it is important for the CPP to stay above 50-70 mmHg and the ICP to stay less than 20 mmHg (Sorrentino et al, 2011), and this is normally regulated by cerebral autoregulation. For example, if one of these waiver from the appropriate level of pressure, the brain will change blood flow to get the pressure to the appropriate level. TBI typically causes significant changes in ICP, CPP, and MAP; more specifically, CPP is normally lowered and ICP is raised. It is this lowering of CPP that has been thought to cause this impairment of autoregulation (Junger et al., 1997). Understanding how TBI affects how the brain autoregulates is necessary in treating TBI. To manage CPP, clinicians would try to either raise MAP or lower ICP, in accordance with the Monro-Kellie principal which states that there is a fixed volume inside the skull. This means that since the skull is incompressible, the changes in MAP or ICP will cause a change in the CPP. Management of ICP and CPP is essential to recovery because if either of the two is off balance serious injury could occur.

The normal cerebral autoregulation is a multifaceted process that involves the myogenic, neurogenic, and metabolic mechanisms performing together (Rangel-Castilla, 2008). Due to changes in pressure above or below a certain threshold, a local reflex is activated which signals these mechanisms to perform contraction or dilation. The myogenic mechanism involves the dilation or constriction of vessels. The neurogenic mechanism controls smooth muscle constriction or dilation, so the neurogenic controls the myogenic. The metabolic mechanism happens in smaller vessels that alter vasomotor response (Rangel-Castilla, 2008).

**Current Methods to Treat Increased ICP:**
Treatment of increased ICP, also known as intracranial hypertension (IH), is something that is not currently completely understood. The general procedure is to lower ICP without causing harm to the patient; however, several different protocols are used from hospital-to-hospital, and even clinician-to-clinician. Some common techniques are controlling the airway or trying to reduce the chance of hypoxia or hypercapnia, which are both cerebral vasodilators, which will lead to increased ICP levels (Su et al., 2011). Another method is to induce hypertension, which will force more blood into the brain raising the CPP (Singh et al., 2006). When the blood brain barrier is still intact, a process known as osmotherapy is used. Administering mannitol by IV will create hypertonic blood, drawing water out of the brain and lowering the ICP. A more invasive approach is drilling small holes in the skull (craniotomies) to relieve pressure. If the ICP increases are uncontrolled by any of these methods, a decompressive craniectomy, removal of a section of the skull, can be used to relieve pressure and make room for swelling and relieve pressure (Shepherd, 2004).

**Techniques of measuring ICP in rats:**

Measuring and understanding ICP is extremely important to finding mechanisms of ICP increases and new methods of ICP management, thereby motivating research on measuring and understanding ICP. One issue that has set back the research being done is the difficulties that arise from trying to reproduce the clinically-seen changes in ICP after TBI in rats, which are the most widely used animal for modeling TBI. There are some studies that show a significant increase in ICP (Rogatsky, 2003) and some that show no significant change following TBI (Gabrielian, 2011). What is seen in current literature is
the use of different techniques of closing the craniectomy site or leaving it open (craniotomy). These techniques are not always explicitly stated in the method section of papers, which further adds to the problem of interpreting ICP rat studies.

**Techniques of measuring ICP that have significant increases in rats:**

In 2003, Rogatsky and colleagues used 27 adult male rats to monitor ICP as a function of injury severity (Rogatsky, 2003). Rats (180-220g) were anesthetized with Equithesin (0.3 mL/100 g). A 6-mm diameter hole was drilled into the parietal bone of the right hemisphere for the sensor probe, and a 3.5-mm hole was drilled in the left parietal bone for the injury mechanism. They placed the probe on top of the brain by removing the dura, and a cannula was inserted into the 3.5 mm hole for fixing the syringe holding device. All of these components were then cemented to the skull with dental acrylic. TBI was then induced by lateral fluid percussion (LFP) brain injury. They separated the rats into four groups A, B, C, and D, and these groups were given different injury severities where groups A, B, C, and D received 2.9, 3.3, 4.1, and 5.0 atmospheres, respectively. Rats were then monitored for ICP for a 5-hour period after TBI was induced. The results showed that there was an initial increase in ICP, then an immediate decrease, followed by another increase at 90 min. At the end of the 5 hours, the ICP levels were 6.75 ± 2.87, 8.40 ± 2.70, 12.75 ± 4.03, 29.56 ± 9.25, respectively for the groups. This paper showed that there is a correlation between trauma severity and ICP increases.

In another, they also used a weight drop (WD) model of TBI, and then measured changes in ICP (Rooker, 2003). Three different groups of ten rats each (390-430 g) were used (sham, mild, and moderate injury). Rats were anesthetized with isoflurane in a
mixture of oxygen and nitrous oxide. In order to injure these rats, a weight was dropped from a certain height onto the skull (mild: 30 cm; moderate: 50 cm). The rats were then prepped for the ICP measurement phase. A small a hole was burred in the right parietal bone, at a point 4 mm from the midline and 2 mm caudal to the bregma suture. An apparatus was placed in the femoral artery until right underneath the skull, and used to guide the ICP probe. The results showed an ICP increase in each of the groups compared with the shams that was significantly higher at every time point tested.

**Techniques of measuring ICP that did not have significant increases in rats:**

Gabrielian and colleagues measured ICP following a variety of insults, including LFP and WD, but reported different results than Rogatsky et al. (Rogatsky, 2003) or Rooker et al. (Rooker, 2003) (Gabrielian, 2011). They used 48 adult males rats (400-500 g), divided into five groups: uninjured, no hypoxia, n=9; LFP only, n=8; WD injury, n=7; WD with secondary hypoxia, n=7, and hypoxia only, n=8. The LFP injury was conducted following anesthesia with 2% of isoflurane in a mixture of oxygen and nitrous oxide and a 5-mm diameter craniectomy. Then a moderate trauma of 2.7 atm was delivered. After injury, an ICP probe was inserted 6 mm below the dura through a cannula into the parenchyma, which was then sealed with bone wax. The WD injury was enabled by releasing a brass weight of 450 g from a height of 2 m through a PVC conduit. The ICP probe was inserted through a burr hole in the left parietal bone. After puncturing the dura, the probe was inserted 6 mm below the dura into the cerebral parenchyma. The results
from this experiment showed no significant increase in ICP for any of the insults when compared to the sham rats.

**Motivation for Study:**

From previous research conducted, there is a discrepancy in the literature as to whether experimental models of TBI produce ICP increases, motivating this research. One way to address this problem is to investigate the difference between a craniotomy versus craniectomy. A craniotomy is removal of a portion of the skull with replacement after the surgery is done. A craniectomy is when a portion of the skull is removed and not replaced. These two terms are used very loosely in current literature with no distinction between the two, craniotomy or craniectomy. Furthermore, the lack of specifying between craniotomy and craniectomy may account for the absence of significant data in some rat ICP studies. The background studies for this research show that there is a discrepancy with both methods, WD and LFP, but for this study, only controlled cortical impact (CCI) will be used. The hypothesis of this study is that the craniotomy group will show more significant ICP increases than the craniectomy group. Therefore, the craniotomy is the appropriate procedure for producing ICP increases in a rat model.

**Methods:**

All procedures involving animals were approved by the Georgia Tech IACUC. We used a CCI injury method to injure male rats and monitor ICP. The CCI device is a pneumatic piston that delivers a reproducible impact to the exposed dura (Pittsburgh Precision Instruments, based on Dixon et al, 1991). Groups were comprised of \( n = 3 \) for craniotomies with injury, \( n = 3 \) for craniectomies with injuries, \( n = 3 \) for craniotomies (sham), and \( n = 3 \) craniectomies (sham). The animals,
300-350 g Sprague-Dawley and 400-500g Wistar rats were anesthetized with a rat cocktail that was given at a concentration of 0.75-1.0 mL/kg. The cocktail consists of 50-80 mg/kg of ketamine, 3-6 mg/kg of xylazine, and 1-3 mg/kg of acepromazine.

For craniotomies with injury, immediate replacement of the bone flap after injury took place. A 6 mm diameter craniectomy was trephined into the skull centered 3 mm right of the sagittal suture and midway between bregma and lambda. The bone flap was replaced with bone cement. Fifteen minutes post injury; a burr hole was drilled on the same side as the injury for the placement of the Samba sensor for ICP readings. The pressure sensor (diameter 0.42 mm) was protected from direct mechanical influence exerted by the surrounding brain tissue with a screw with a hole in the center (Krave et al., 2005). The sensor was then inserted into the brain tissue 4-6 mm below the skull surface (Jennische et al. 2008). The animal was continuously monitored over a 30 min period because ICP is expected to increase over time.

For craniotomy shams, the bone flap was replaced after the craniectomy. A burr hole was drilled fifteen minutes after skull section is replaced. Everything followed the same procedure as the craniotomies with injury after this.

For craniectomies, the same procedure as the craniotomy procedure was performed, except no replacement of bone flap immediately after injury. The sensor was inserted on the opposite side of the skull, and the rest of the procedures from
the craniotomy with injury trials were done. The sham craniectomy group followed
the same procedure as well except with the injury of the CCI device.

In order to measure tissue edema, which is expected to correlate to ICP (Donkin et
al., 2009), the wet-dry method (O’Connor et al., 2006; Donkin et al., 2009) was used
on all four groups. This involved decapitating the rat, removing the brain, weighing
the brain, and then letting the brain dry for 72 hrs at a temperature of 100 degrees
Celsius. The following formula is then used to obtain a brain-water content:

\[
\text{water} = \frac{(\text{wet weight} - \text{dry weight})}{\text{wet weight}} \cdot 100.
\]

If edema is found, it may account for the
increase in ICP. The larger amounts of edema should correlate to a higher ICP.

**Results:**

After all the trials were completed, there were three Injured Craniotomies, three
Injured Craniectomies, one Craniotomy Sham, and one Craniectomy Sham.

**Craniotomy Injured:**

For the craniotomy section, the values for Trials 1, 2, and 3 were 5.04±0.85,
7.43±0.97, and 7.24±0.94 respectively. This correlated to a very controlled, nearly
linear increase as can be seen in Figure 1. Out of the three trials, two of them (Trial
2 and Trial 3) increased and one decreased (Trial 1).

The edema study gave promising results. For trial 1, there was a 72.6% wet brain
percentage. This corresponds to an 1892 mg wet brain and a 518.2 mg dry brain
weight. There was a 3.49 m/s impact at a 2mm depth. For trial 2, there was a 78.2%
brain water percentage. This corresponds to a 2284 mg wet brain and a 497 mg dry
brain. There was a 3.7 m/s impact at a 2mm depth. For trial 3, there was a 78.1 % wet brain percentage. This corresponds to a 2190.1 mg wet brain and a 479.9 mg dry brain weight. There was a 3.59 m/s impact at a 2mm depth.

![Figure 1: Craniotomy Injured Results](image)

**Craniectomy Injured:**

For these procedures, there was no real correlation between the three trials. The values for Trials 1, 2, and 3 were 3.83±0.51, 6.81±1.35, and -0.076±6.31 respectively. For these trials as can be seen in Figure 2, Trials 1 and 2 show very inconsistent data points. Trial 3 showed the most inconsistency by decreasing by 12, which is the largest change seen in all of the studies.

The data from the edema study was calculated next. For trial 1, there was a wet brain percentage of 76.5%. This corresponds to a 1928.7 mg wet brain and a 451.4 mg dry brain. There was a 3.48 m/s impact at a 2mm depth. For trial 2, there was a wet brain percentage of 76.0%. This corresponds to a 1989.9 mg wet brain and a
477.3 mg dry brain. There was a 3.13 m/s impact at a 2mm depth. For trial 3, there was a wet weight of 1443.8 mg and a dry weight of 296.6 mg, which equals a brain water percentage of 79.5 %. There was a 3.50 m/s impact at 2mm depth.

![Graph showing ICP over time for three trials](image)

**Figure 2 Cranectomy Injured Results** This figure shows the results from the three cranectomy injured trials.

**Craniotomy Sham:**

For this trial, the data gave an average reading of 7.33±0.30. This gave an average difference of 0.33 mmHg for this trial. This data gave very consistent changes in ICP. This can be seen in Figure 3.

For the edema study of this trial, there was 79.3 % water content in the brain. This corresponds to a wet brain weight of 2004.5 mg and a 414.6 mg dry weight. There was a 3.8 m/s impact on this animal.
**Cranietomy Sham:**

The data from this trial gave an average of 4.90±1.18. The data can be seen in Figure 3.

For the edema study of this trial, there was a wet weight of 1443.8 mg and a dry weight of 296.6 mg, which equals a brain water percentage of 79.5 %.

*Figure 3 Sham Trials of Craniotomy vs Craniectomy*
Figure 4 Comparison of average Craniotomy, Craniection, and Sham results

Statistical Analysis:

ICP Measurements Analysis:

A two-way ANOVA was performed on the data within each group. For the Craniectomy Injured, there was a p value of 0.33 which means there is no significance because it is greater than 0.05. The Craniotomy Injured rat trials had a p-value of 0.89. This means neither group showed significance to one another.

A repeated-measures ANOVA was performed to compare injured versus sham rats. Then the craniotomy injured trials were compared to the craniectomy injure. This resulted in a p-value of 0.587. There was no significant difference in these.

Edema Measurement Analysis:

A one-way ANova was used to compare all the injuries with all of the shams. The p-value was 0.696 meaning there was no significant difference between all the groups. Then the craniotomy injuries were compared to the craniectomy injuries. This p-value was 0.656, meaning no significant difference was observed. Then the craniectomy injured was compared to the craniectomy sham, which gave a p-value of
0.426, meaning no significance. Then the craniotomy injured trials was compared to the craniectomy shams. This p-value was 0.541. There was no significant difference observed in any of the statistical analysis of this section.

Discussion:
The topic of craniotomy and craniectomy is something very important in the world of TBI research because it could lead to better techniques for research a very serious problem. This study tries to see the differences between the two types of procedures to determine which is the best option for rat studies. Since the number of trials was so small no real conclusions can be taken from this study. However, there are some trends that can be noticed in the data. The craniotomy procedure shows the most consistency in its results. As shown in Figure 1, Trial 1 consistently went down linearly. The Trial 2 and Trial 3 each increased consistently. Just from this data one could conclude that the craniotomies provide a much more stable and controlled model. The craniectomies on the other hand were not consistent a lot as seen in Figure 2. There was never a real increase or decrease in the results. Even the craniectomy sham shows very uncontrollable and unstable readings when compared to the craniotomy sham as seen in Figure 3. Further study still needs to be done to prove the hypothesis that craniotomies are more reliable for the rat testing model and show the higher ICP readings, but this study can at least show that out of the trials done the craniotomies show the most stable and consistent data. As seen in Figure 4, the craniotomy followed the manner in which the sham behaved. The
hypothesis would still be that the craniotomy model should give more reliable data about TBI in rats.

There have been similar studies including the one mentioned in the literature review section of this paper. It has been shown that after injury ICP can be discovered using a rat model. In this study, the researchers closed up any open holes with bone cement that allowed them to create an airtight seal (Rogatsky, 2003). This would be comparable to a craniotomy. In this study, the researcher was able to get significant increases in ICP (Rogatsky, 2003). This study shows that with craniotomies, the data is more reliable because it has the most constant and linear changes over time.

In addition to this study, there is another study that used the craniectomy method (Gabrielian, 2011). In this paper, the researchers were not able to find significant increases while using this rat model. They were only able to show significance when intracranial hemorrhage was present (Gabrielian, 2011). This study shows the same non-significance between trials of the same method type.

The edema study also gave no conclusive results. Previous studies have found that the percent of water in the brain after TBI were close to 80% (Donkin, 2009). The average for the craniotomy injuries were 76.2%, the craniectomy injuries gave 77.3%, and the shams had an average of 79.3%. These were not conclusive enough to draw a conclusion.

For these reasons, further research is still needed. The main reason is because there were many limitations in this study. The first one is time. In the major previous studies (Gabrielian, 2011 and Rogatsky, 2003), there was a longer time for
each trial. They both use 4-5 hours of testing. This was impossible because of limitations on time available to perform trials. There were also the limitations of money, which did not allow for more rats of the same size and type to be used throughout this entire study. The rats were changed to a different rat type in the middle of the study so the data could be skewed from this. There was also far more rats in each study. The previous studies used 48 rats (Gabrielian, 2011) and 27 rats (Rogatsky, 2003). These rats were also kept consistent throughout the procedure, which includes type and weight.

In conclusion, this study shows that rats with craniotomies have much more consistent data on time. As one can see in Figure 1 and Figure 3, the craniotomy trials show a much more consistent reading meaning there isn’t a lot of bizarre outliers. This study needs to be continued allowing for more consistency with the rats’ size and weight, and then there needs to be a higher sample size. This will allow for a conclusion to be made about craniotomies versus craniectomies.
Resources:


Tyler, K.L., 2004. Herpes simplex virus infections of the central nervous system: encephalitis and meningitis, including Mollaret's. Herpes 11 (Suppl. 2), 57A–64A.