The Dana Foundation's "Design a Brain Experiment" Competition

2017 Second Place (tied)

"Potential Treatments Against Cortical Spreading Depression to Prevent the Secondary Effects of Post-Traumatic Brain Injury"

By Benjamin Myers

Bard High School Early College II, New York

Lay Summary (provided by the Dana Foundation):

Each year in this country approximately 2.5 million people suffer a Traumatic Brain Injury (TBI), including concussions, blows to the head or any other impact that disrupts normal cognitive function for any period of time. These primary injuries can produce many secondary effects, such as difficulty thinking, remembering, and concentrating, as well as physiological effects such as Cortical Spreading Depression (CSD). Upon CSD, there is a sudden and migratory depolarization of all neuronal cells (including neurons, glia and other astrocytes) in the area of impact, essentially creating a state of 'paralysis' where the cells are unable to function properly. CSD can worsen the secondary effects of TBI and cause lasting damage to neurons. Benjamin is seeking a means to

decrease the speed, intensity and duration of a CSD to enable neurologists to intervene before CSD causes lasting damage to neurons.

CSD interferes with the neuronal homeostasis of the brain through a massive increase in the amount of energy required to maintain homeostasis, and produces temporary dispersal of the substrates (such as glycogen and lactate) that power ATPase ion pumps. Benjamin considers the most effective way to target CSD would be through the complete and rapid restoration of neuronal homeostasis via restoration of the substrates needed to power these pumps. The pumps primarily rely on substrates such as Glucose, Oxygen and other polysaccharides. Just as hospitals created more efficient anesthetics that target specific brain areas based on their agonist/substrate base, such as short versus long- term anesthesia, Benjamin looks for a more efficient substrate combination to enable ATPase ion pumps to rapidly reestablish homeostasis.

His experiment in mice is designed to determine which substrate (glycogen, sucrose, or insulin) when injected into the body most effectively and efficiently reestablishes homeostasis post-CSD. He finds that glycogen is the best of the three.

I. Purpose

Each year in America alone, approximately 2.5 million people suffer a Traumatic Brain Injury (TBI), including concussions, quick or unanticipated blows to the head, or any other impact that disrupts normal cognitive function for any period of time (1). These traumatic primary injuries can cause a variety of secondary effects, such as difficulty thinking, remembering, and concentrating, as well as physiological effects such as Cortical Spreading Depression (CSD) (2). Through the use of administered polysaccharides I aim to combat these secondary effects of TBI's and prevent lasting nerve damage.

II. Question

Which substrates (glycogen, sucrose, or insulin) when injected into the body are the most effective in reestablishing homeostasis post CSD caused by a TBI?

III. Background

Cortical Spreading Depression (CSD) is a sudden and migratory depolarization of all neuronal cells (including the neurons, glia and other astrocytes) in the area of impact (2). Thus, it essentially puts the neurons into a state of 'paralysis' where they are unable to function properly, which in turn can worsen the secondary effects of TBI's and cause lasting damage to the neuron (3). To be able to find a mechanism to decrease the speed, intensity and duration of a CSD on a neuron would allow doctors and neurologists to combat secondary neuron damage before it causes lasting damage.

As CSD interferes with the neuronal homeostasis of the brain through a massive increase in the amount of energy required to maintain homeostasis in addition to temporary dispersal of the substrates (such as glycogen and lactate) which power the ATPase ion pumps, the most effective manner to target CSD would be through the complete and rapid restoration of neuronal homeostasis via restoration of the substrates needed to power these pumps (4). Homeostasis is very delicate mechanism and cannot be interfered with for long, therefore, to find the most efficient substrate to power the ATPase ion pumps would allow homeostasis to be rapidly restored.

The specific ATPase ion pumps that are involved in establishing and maintaining homeostasis are primarily reliant on substrates such as Glucose, Oxygen and other polysaccharides; this knowledge, in fact, has been used to create more efficient anesthetics in hospitals that can help provide the exact type of substrate needed for a certain neurological condition (5). For example, different anesthetics which target different sections of the brain will require different agonists and substrates in order to affect only that singular part of the brain. Additionally, these various anesthetics have differing effects based on their agonist/substrate base, such as short versus long term anesthesia, increased caloric intake, and hallucinations (5). Therefore, by following
the model of these anesthetics, I hope to be able to find a more efficient substrate combination that would allow the ATPase ion pumps to reestablish homeostasis more rapidly.

Methodology:

Using the methods described in Von Baumgarten et. Al (6), I will divide thirty mice into three experimental groups (n = 8) and one smaller control group (n = 6). The experimental groups will receive the experimental substrate combinations post CSD induction, while the control group will not receive any

substrate infusion post CSD induction. CSD induction will be done according to the procedures described in Von Baumgarten et. Al (6), through the application of a surgical drill with a revolution of 6 m/sec for 150 ms to the right parietal cortex at the same time as KCL injection to further depolarize the neurons At the same time, I will apply two probes to measure EEG levels. Homeostasis will be determined to be 'reestablished' when all readings reach normal, pre-injury levels. The animals (previously anesthetized following the halothane 4% procedure found in Baumgarten et. Al (6)) would then be incubated for thirty minutes to allow for proper recovery.

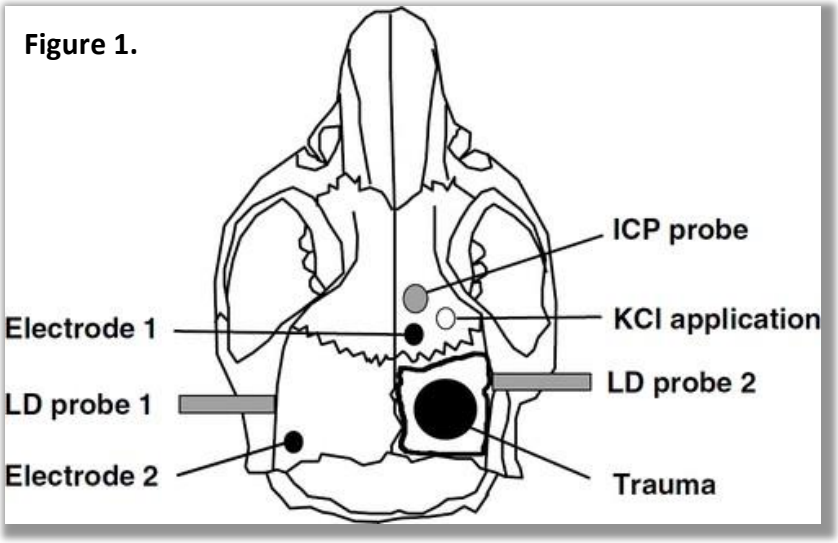


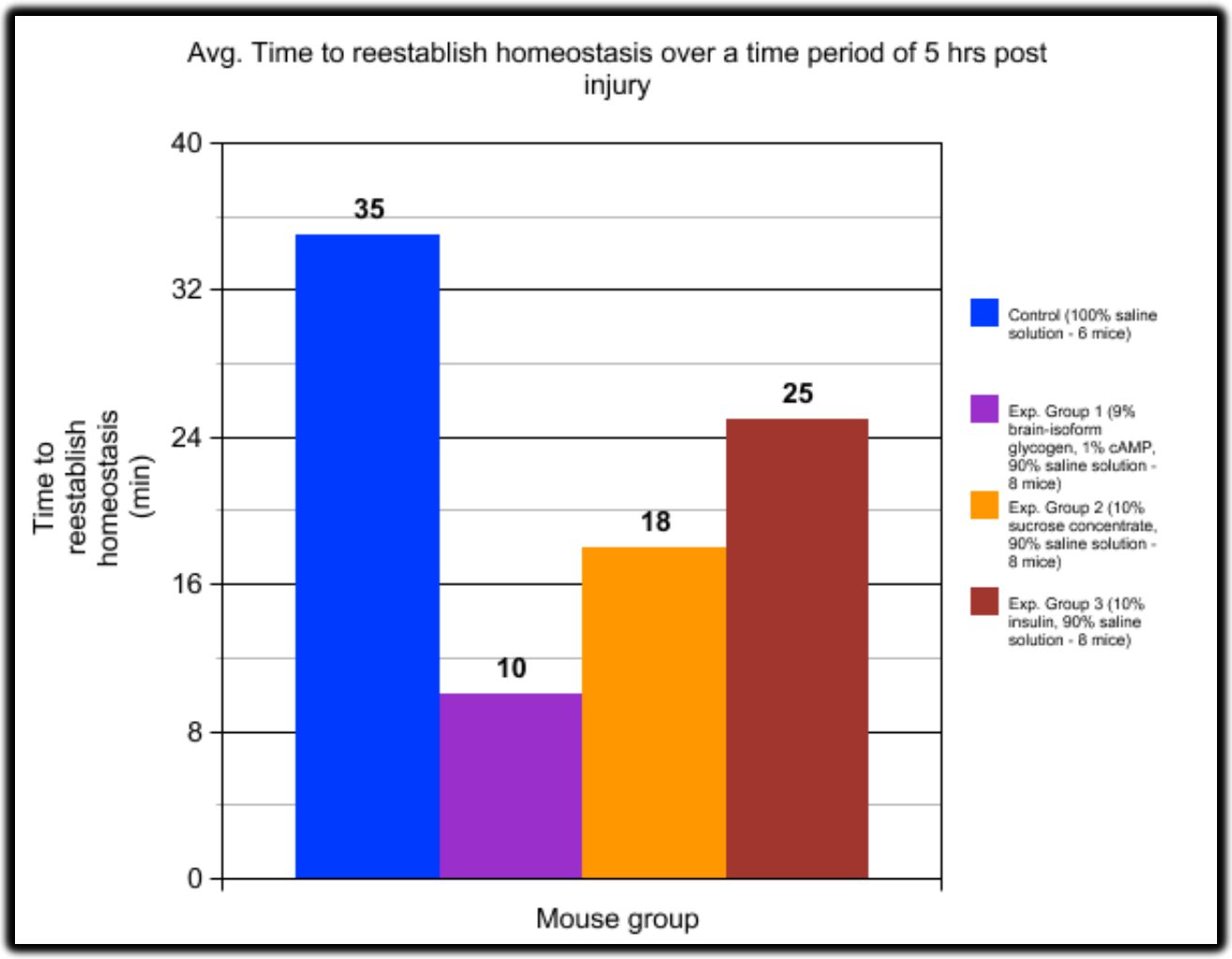
Figure 1: In this image, the black square represents the removal of the cranial skeleton to allow access to the trauma area, defined as the black circle. The two electrodes allow for DC measurement, which in turn amplifies the input to the EEG allowing for easily facilitated readings. The two LD probes are the locations of the probes for the measurements for intracranial pressure. The empty circle is where the KCl is injected into the brain to further depolarize and spread the CSD (6).

Table 1. Experimental Design

Group	Treatment (given intravenously 30 minutes post injury)
Control	100% saline solution
Exp. Group 1	9% brain-isoform glycogen (7) 1% cAMP (8), 90% saline solution
Exp. Group 2	10% sucrose concentrate, 90% saline solution
Exp. Group 3	10% insulin, 90% saline solution

The experimental design is summarized in **Table 1**, which delineates the experimental group and the intravenous solution specific for each group. Measurements of the EEG and Intracranial pressure, along with the Doppler will take place continuously for up to 5 hours post-injury, as well as 1hr, twenty and ten minutes pre-injury.

Results:



Understanding the overall time for a control group to reestablish homeostasis after the initiation of a single CSD (additionally understanding that serval CSD's may take place in a single hour) to be approximately 35 minutes (6), the overall time for the glycogen group to reestablish homeostasis will be greatly decreased (by approximately 350%) to a mere ten minutes. The extended time for the sucrose group indicates that while it is a simpler molecule (10) and should be easier for the ATPase ion pumps to break down and use as a form of energy, it cannot provide the same amount of high-potential energy that glycogen can produce. However, as it does have a reduced time with respect to the insulin experimental group. This is possibly due to the fact that the insulin group has to go through the additional process of activating the MAPK and PI3K pathways, which *then* help to work to reestablish homeostasis.

Should the glycogen turn out to have no effect (essentially disproving my hypothesis) I will then have to use a different type of high-energy organic molecule, such as a different type of polysaccharide. Should that additionally be ineffective, then I must attempt a different type of molecule, possibly something non-organic that has been proven to release high amounts of energy in the generation of ATP.

Conclusion:

As CSDs relating from injuries such as TBIs happen to approximately 2.5 million Americans per year, it is absolutely pressing that neurologists be able to combat this threat and prevent any continuing post-traumatic injuries. This study aims to be able to combat these CSD's through the rapid reestablishment of homeostasis induced through the presence of various substrates proven to provide energy needed for ATPase ion pumps. By isolating the most effective organic molecule to 'jump-start' the reestablishment of homeostasis, neurologists can thus be able to provide more effective and targeted treatment for their patients. This project isolated Glycogen, Sucrose and Insulin and tested their relative effectiveness in reestablishment of homeostasis. I eventually discovered that glycogen proves the most effective in this task.

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