Investigating Neurovascular Coupling During Brain Seizures After Hypoxia-Ischemia

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**Introduction:**

There is a long record of experimental evidence that has shown that cerebral blood vessels are uniquely associated developmentally and functionally with neurons and glia in the brain\(^1\). The mechanism is still not fully understood but most believe it is triggered vasoactive neurotransmitters that are released during neural activity causing the cerebral blood vessels to locally contract or dilate depending on the energy demands of the local brain tissue in a process called neurovascular coupling\(^1\).

Hypoxic-ischemic brain damage occurs when there is not enough oxygen or glucose being delivered to brain damage\(^2\). The lack of oxygen causes decreased production of high energy phosphates in brain tissue leading to several pathways that disrupt cell integrity and eventually cause cell death\(^2\). When hypoxia-ischemia is induced in piglets it leads to periods of seizure activity in the brain. This creates an easily recognizable spiky pattern in the electrical activity of the brain on an ambulatory electroencephalography (aEEG) readout which is shown in figure 1. An aEEG measures the electrical activity in the neurons of the brain by measuring the changes in voltage at points along the scalp\(^3\).

Measurements of electrical activity in a piglet’s brain and the concentration of total, oxy and deoxy-hemoglobin were collected simultaneously using aEEG and near-infrared spectroscopy (NIRS) respectively. The objective of this experiment was to see if normal
neurovascular coupling was maintained during hypoxia-ischemia induced brain seizure activity and to see if there was a characteristic vascular response to an individual peak of brain activity (refer to figure 1) using a piglet model. Theoretically, each pulse of brain activity will create ionic currents which will cause an increase in the concentration of total and oxy-hemoglobin and a decrease in deoxy-hemoglobin because of neurovascular coupling (Neurovasc). Therefore, the hypothesis is that there will be an increase in the concentration of oxy-hemoglobin and total hemoglobin and a decrease in deoxy-hemoglobin corresponding to an individual seizure event.

Theory:

aEEG:

An aEEG measures the amount of electrical activity in the brain’s neurons by measuring changes in voltage along the scalp. As currents are induced in the brain’s different neurons the voltage values change indicating an increase or decrease in total electrical brain activity\(^3\). After hypoxia-ischemic brain damage there are usually seizures in brain activity resulting in sections of aEEG data that oscillate with a high frequency between a normal baseline voltage and an elevated voltage. Seizures in brain activity are spontaneous and can occur in any pattern but figure 1 shows a sample of the aEEG data from the seizure that was studied in this experiment. Post hypoxia-ischemic brain seizures were detected in piglets using aEEG then once the seizures were found NIRS data from this part of the experiment was analyzed.

Modified Beer-Lambert Law:

The classical Beer-Lambert law is used for non-scattering mediums but brain tissue will scatter near infrared light so the modified Beer-Lambert law must be used instead\(^4\).

\[
A = (\lambda) \ast c \ast L + G(\lambda) \quad (Eq. \ 1)
\]
In this equation $A$ is absorbance, $L$ is the path length, $c$ is the concentration of the chromophore, $(\lambda)$ is a wavelength dependent extinction coefficient and $G(\lambda)$ is the contribution of the attenuated light due to scattering$^4$. The modified version of the law relies on the simplifying assumptions that the media being imaged is homogenous and that there is high but constant scattering in the throughout$^4$. With this assumption $G$ is not known but it stays constant so changes can be found by subtracting $G$ out of the absorption data but absolute concentrations cannot be found$^4$. This is considered a valid assumption because the absorption coefficient is affected much more by changes in oxygenation and concentration then it is by the scattering coefficient$^4$.

**NIRS:**

NIRS is an imaging technology that uses 600 to 900nm light to monitor the changes in state of biological tissues, particularly the changes in the concentration of total, oxy and deoxy-hemoglobin$^5$. NIRS was ideal for this experiment because it provides good temporal resolution when used to measure tissue oxygenation which makes it ideal for looking at fast oscillations related to normal physiological functions$^4$. Continuous wave NIRS devices make the simplifying assumption that there is high and constant light scattering throughout the tissue which allows them to measure changes in concentration of total, oxy and deoxy-hemoglobin$^5$.

Figure 2 shows the curved path caused by scattering that light is assumed to travel after leaving the source. Figures 4, 5 and 6 are normalized to a baseline, this means that the first ten cells were averaged and subtracted from the rest of the data in the set. This way the
first ten cells represent the baseline concentration and the rest of the graph shows the change from baseline for oxy, deoxy and total hemoglobin concentration.

**Neonatal Hypoxia-Ischemia:**

Hypoxia-ischemia occurs when not enough oxygen is delivered to the brain, neonatal hypoxia-ischemia is a specific case where insufficient oxygen is delivered to an infant’s head during birth\(^2\). High energy phosphates are depleted quickly since the lack of oxygen decreases their production, this causes ATP dependent ion pumps to fail causing intracellular accumulation of sodium, calcium and water\(^3\). Elevated levels of calcium cause the activation of phospholipases, proteases and nucleases which degrade the lipids, protein and DNA of the cell respectively\(^2\). Glutamate also accumulates in the inter-synaptic region since it is normally removed by ATP dependent pumps and the resulting activation of glutamate receptors causes more calcium ions to enter the cells\(^2\). Neonatal hypoxia-ischemia also increases concentrations of nitric oxide and iron which are both strong oxidants causing oxidative damage in the infants’ brain\(^2\). Neonatal hypoxia-ischemia still affects about 0.2% of births in developed nations and sixty percent of affected infants die while about twenty five percent of survivors have long-term brain damage\(^3\). Piglets are sometimes used as a model for infants affected by neonatal hypoxia-ischemia, since this experiment uses a piglet model it will allow us to verify how realistically a piglet models neonatal hypoxia-ischemia.

**Methods:**

The NIRS and aEEG data used for this project had already been collected from a piglet on May 5\(^{th}\) 2011. For my project one of the programs I used was provided by my supervisor, it allowed users to search through the aEEG data and make graphs of specific segments by picking
a starting and stopping time. The program outputted two graphs, one was the aEEG data and the other one was the transistor-transistor logic (TTL) signal, which indicates whether or not the NIRS machine was collecting data. This program was used to look for periods of oscillatory seizure activity, eventually a suitable seizure was found starting at approximately 12:06pm and lasting until approximately 3:39pm.

The NIRS machine was kept off and then turned on at certain times to monitor the changes in blood flow after injections of indocyanine green (ICG). As a result all of the aEEG data is contained in one file while the NIRS data from the same data collection is contained in multiple files. After looking at journals from the data collection it was determined that the NIRS data corresponding to the time of seizure activity was collected in between the fourteenth and fifteenth ICG injections. This revealed what file the NIRS data of interest was in but the start of both data sets needed to be synchronized so that the neurovascular response to an individual seizure response could be analyzed. To find the start time of the NIRS data the aEEG program was used to make graphs of the aEEG and TTL signal from the times around the start of the seizure activity. The TTL signal was used to find the exact time between the start of the aEEG data and the start of the NIRS data from between the fourteenth and fifteenth ICG injections.

![Figure 3: The TTL signal is on the right it gives the exact start time for NIRS data acquisition, this technique was used to find how to synchronize the start of the two datasets.](image)
Figure 3 shows the aEEG and TTL signal for the same time period, when the TTL signal is at zero the NIRS is off and when it is at approximately five the NIRS is on. The journal was used to find the estimated starting time for the NIRS data file and then the TTL signal was used to find that the NIRS data file started exactly 177.0087 minutes after the start of the aEEG data acquisition. A code had to be written which allowed users to easily search through the NIRS data so that a link between neural activity and blood flow could be established.

The main idea of the new code was that users could isolate individual events in seizure activity using the aEEG program, then once an event was isolated the exact same times used to isolate the event could be entered into the new code. The code would then subtract 117.0087 minutes from the user inputted start and stop times and multiply the results by sixty to convert to seconds. Graphs of the changes in concentration of total, oxy and deoxy-hemoglobin were then created.

The aEEG program was used to find exact start times for the first ten individual seizure events then the NIRS program was used to find a nine second section of data for each event. The data from each individual event had a lot of noise in it but it still did show some evidence of a pattern. To try to remove some of the noise the first ten responses were averaged together, this way there was one graph for total, oxy and deoxy-hemoglobin where each data point was the average of ten values from the ten first seizure events. The average responses from this experiment were then compared to both a normal neurovascular response and the neurovascular response from neonates who have suffered from hypoxia-ischemic brain damage.
Results:

Figure 4 represents the change in oxy-hemoglobin over a nine second period in response to one seizure event. Each data point is the average of the values from the first ten seizure peaks.

![Average HbO Response](image)

Figure 4: The graph is normalized to baseline so the y-axis shows the change in concentration brought on by the seizure event, not the absolute concentration. The error bars are the standard error (σ/\(n\)^0.5) of the mean of the ten samples.
Figure 5 shows average change in the concentration of deoxy-hemoglobin in response to a single seizure event. This graph is also averaged to baseline so the y-axis shows only changes in concentration and not the absolute concentration.

![Average Hb Response](image)

Figure 5: The error bars on this figure are the standard error of the mean of the ten samples.
Figure 6 shows the average change in total hemoglobin brought on by an individual seizure event.

![Average HbT Response](image)

**Figure 6**: This graph is averaged to baseline so the y-axis shows changes in concentration and not absolute concentrations. The error bars on the graph represent the standard error of the mean.

**Discussion**:

Even in the averaged data sets for the changes in total, oxy and deoxy-hemoglobin a lot of noise still shows up. Despite the noise, a general pattern is still observable for total and oxy-hemoglobin but the averaged response of deoxy-hemoglobin concentration does not show much of an increase or decrease, it stays pretty close to the baseline. If the concentration data from
figures 4, 5 and 6 are all put onto the same graph the complete response to an individual seizure event can be analyzed and compared to a normal response or a pathological case.

![Averaged Response to Seizure Event](image)

Figure 7: This graph shows the averaged response to a single seizure event for total, oxy and deoxy-hemoglobin. The error bars have been removed for clarity, please refer to figures 4, 5 and six to see the error bars for oxy, deoxy and total hemoglobin respectively.

Figure 7 is a representation of the complete neurovascular response to a single hypoxia-ischemia induced seizure in a piglet. There is a clear increase in the concentrations of total and oxy-hemoglobin which supports the hypothesis and shows that an increase in neuronal activity causes an increase in blood flow to the active area of the brain. Intuitively, an increase in the oxy-hemoglobin concentration should correspond to a decrease in the deoxy-hemoglobin concentration as oxygenated blood from arteries washes deoxygenated blood out the veins back to the heart\(^7\). Instead of the expected drop in concentration for deoxy-hemoglobin the values do not increase or decrease very much from baseline. Figure 8 shows an idealized version of the
normal neurovascular response, the figure is from a lecture from an introductory PhD course about neurovascular coupling. The gray bar at the bottom of the figure represents the stimulation of a single whisker on a rat causing a small burst of activity in the brain. In the normal response the blood flow increases to the brain in response to the stimulation and the concentration of deoxy-hemoglobin drops as the deoxygenated blood is replaced with oxygenated blood. This way the brain cells are able to maintain a constant supply of oxygen and sucrose even when there is more metabolically demanding neuronal activity.

Figure 8: This figure shows the idealized neurovascular response to an increase in neuronal activity.

The main way that the neurovascular response differs from the averaged response collected from the piglet is that there is no drop in the concentration of deoxy-hemoglobin in the piglet. The brain has a high oxygen metabolism so it could mean that as deoxygenated blood is being pumped out of the brain the concentration of deoxy-hemoglobin is maintained by very fast conversion of oxy-hemoglobin to deoxy-hemoglobin through metabolic processes. It may also mean that there was just too much noise in the data to see the expected drop in deoxy-
hemoglobin concentration. The comparison between figures 7 and 8 show that neurovascular coupling is still present during seizure activity after hypoxia-ischemia induced brain damage but the process is inhibited so it is not fully effective.

Since piglets are used to model neonatal hypoxia-ischemia it is also useful to compare the neurovascular response in the piglet to the neurovascular response in infants who have suffered from hypoxia-ischemia induced brain damage. If the piglet and the infant have a similar neurovascular response to an individual seizure event it will further validate the piglet system for modelling neonatal hypoxia-ischemia. Figure 9 shows the neurovascular response to a single peak of aEEG activity in infants who have suffered neonatal hypoxia-ischemia.

Figure 9 shows the concentration changes in total, oxy and deoxy-hemoglobin after a single peak of aEEG activity represented by the horizontal gray line starting at 0 seconds.

Figure 9 starts off with deoxy-hemoglobin rising in concentration because of the metabolically demanding aEEG burst starting at zero seconds. Then total and oxy-hemoglobin increase in concentration as the neurovascular response starts and deoxy-hemoglobin starts to
drop in concentration as oxygenated blood flows into the brain and replaces the deoxygenated blood. It is less smooth than the normal neurovascular response shown in figure 8 and the concentrations of total and oxy-hemoglobin do not start to increase until about three or four seconds after the start of the EEG burst. In the normal response the concentrations start to increase approximately one or two seconds after the start of the neuronal activity. Figure 9 is closer to the averaged neurovascular response collected from the piglet than the normal neurovascular response. There is a short period near the beginning of the data in figure 7 where the concentrations of total, oxy and deoxy-hemoglobin are generally rising, then the concentration of deoxy-hemoglobin starts to drop while the total and oxy-hemoglobin concentrations continue to increase just as in figure 9. There is still little evidence of a pattern in the concentration data for deoxy-hemoglobin collected from the piglet. Aside from this the increase in total and oxy-hemoglobin is pretty similar between the data collected from the piglet and the data collected from the pathological infant. This provides good evidence that the piglet model is an effective approximation of neonatal hypoxia-ischemia.

There are a couple sources of error in this experiment which may have obscured the data to the point where the drop in deoxy-hemoglobin concentration could not be seen. To remove noise from the neurovascular responses to individual seizure events nine second data sections were taken from each of the first ten individual events and the values at each time point were averaged together. The individual seizure events are not uniform in shape so it was difficult to synchronize the start of the ten peaks together. I tried to just enter the starting time as close to the start of the ascending part of the seizure but it may have been more accurate to use a uniform starting point. This could be done by taking data starting one second before the highest value on the peak and going until eight or nine seconds after the event has ended. The only other
significant source of error that could have happened when the code was written is if the TTL signal was read wrong. This would mean that the start of the NIRS data was not properly synchronized with the start of the aEEG data and all of my data would be shifted by the same amount of time, obscuring the true response to a single seizure event. The synchronization time between the NIRS and aEEG data was extremely important to the project so extra care was taken to make sure it was accurate, therefore it is likely not a large source of error. The data may have less noise in it if the starting points for the individual seizure events were better synchronized using the process outlined above.

**Conclusions:**

The objective of this experiment was to see if there was a characteristic neurovascular response to an individual spike in neuronal brain activity during hypoxia-ischemia induced brain seizure activity. Hypoxia-ischemia was induced in a piglet then it was monitored with an aEEG and NIRS machine to collect data on the electrical activity of the brain as well as the concentration changes of total, oxy and deoxy-hemoglobin. A matlab code was written to synchronize the start of the aEEG data with the start of the NIRS data and blood concentration changes corresponding to the first ten individual peaks were averaged together. The averaged responses showed that the concentration of total and oxy-hemoglobin both increased but the concentration of deoxy-hemoglobin stayed relatively close to baseline. The hypothesis of the experiment was that normal neurovascular coupling would cause an increase in total and oxy-hemoglobin and a decrease in deoxy-hemoglobin\(^7\). The hypothesis must be rejected because the concentration of deoxy-hemoglobin does not change much from baseline but the predicted
change in total and oxy-hemoglobin was observed in the piglet so there is still good evidence that neurovascular coupling is maintained during seizure activity brought on by hypoxia-ischemia. The data collected from the piglet was similar to the data collected from infants suffering from hypoxia-ischemia induced seizures in brain activity but there was still a much more recognizable pattern in the deoxy-hemoglobin concentration data from the neonate that was not seen in the piglet neurovascular response. More material must be analyzed before meaningful conclusions can be made as this experiment only investigated one episode of hypoxia-ischemia induced brain seizure in one piglet. If the experiment was performed again a more accurate way of synchronizing the start of the individual seizure events may reduce the noise in the data and make the curves smoother. This experiment provided good evidence that neurovascular coupling is maintained during seizure activity brought on by hypoxia-ischemia. It also showed that a piglet model is a good approximation of neonatal hypoxia-ischemia but more data must be analyzed from different periods of seizure activity and in different piglets compile more evidence.

References:


