

Oxygen Absorption in Hemoglobin and Brain Activity Measurement

Quantitation of Oxygen Concentration

NSF Summer Undergraduate Fellowship in Sensor Technologies

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ABSTRACT

Many studies today are executed with the goal of inventing a non-invasive method to measure blood oxygen levels in the brain. The current methods include taking blood samples during surgery or using a pulse oximeter. Yet, neither of these methods yields direct or immediate data about brain oxygen levels. The goal of this project was to measure the concentration and saturation of hemoglobin with oxygen and associate this with how Near Infrared Light Spectroscopy (NIRS) can be used in a non-invasive way to measure brain activity. The protocol of this experiment was to use the Clarke oxygen electrode to measure absorption levels of oxygen in yeast. After executing this experiment many times, the same method was to be transferred to hemoglobin, an animal's brain, and finally the human brain. The Cognosensor (brain imager) invented by Dr. Britton Chance, which uses NIRS to correlate changes in oxygen levels to measure brain activity, was used during the hemoglobin experiment. During all of these experiments, levels of oxygen concentration were measured and plotted respectively. The results of these experiments were used to correlate the methods used on the aforementioned brain imager. This information could yield to developing a low-cost, non-invasive method to measuring oxygen concentration and saturation of hemoglobin in the brain.

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1. Introduction

The human brain is one of the few components in the body of which we know relatively very little. This is largely due to that fact that the human brain is very sensitive, and we cannot invasively measure changes in its activity. Therefore, the ability in the present day to accurately predict a particular cognitive function given a pattern in the change of blood volume levels is limited. The current methods for measuring cognitive function are very innovative. Yet, many of these techniques require the use of large or invasive machinery in addition to immobilization of the subject. Also, the equipment costs of many of these techniques further puts their use outside the realm of most environments. [1]

There are methods available today for measuring cognitive function such as functional magnetic resonance imaging (fMRI), electroencephalogram (EEG), and positron emission tomography (PET). Yet, Near Infrared Spectroscopy (NIRS) is non-invasive, and relatively cheap when compared to the aforementioned methods.

Near Infrared spectroscopy (NIRS) is a safe, non-invasive way for measuring hemodynamic response using an absorption band of deoxyhemoglobin (Hb) and oxyhemoglobin (HbO₂) between wavelengths of 700-900 nm. In the 1980s, a single unit, stand-alone NIRS was made available, but the application of Near-Infrared spectroscopy was focused more on chemical analysis. It became a much more powerful tool for scientific research in more fields such as physics, physiology, and medicine in the early nineties, with the introduction of light-fiber optics and monochromator-detector developments.

Dr. Britton Chance's laboratory at the University of Pennsylvania School of Medicine has created a brain imager which employs Near Infrared Spectroscopy to measure changes in oxygen levels. Furthermore, Dr. Chance and his team have designed a remote, handheld sensor device which is simple, cheap, and is used to detect changes in the blood volume levels of the brain. [1] This study focuses on NIRS, how it is used to quantitate oxygen concentration levels in hemoglobin, and how hemoglobin serves as a biosensor of brain functional activity.

2. Background

Near Infrared Spectroscopy is a technique used for safely measuring the changes in blood volume during cognitive function for *in vivo* experiments. It is an innovative approach to cognitive function analysis and has recently been picking up in popularity because it is non-invasive, easy to implement, and relatively cheap. It is used in a variety

of procedures including tissue density monitoring, cancer detection, oximetry, and brain function detection. For the past ten years, it has been used for *in vivo* measurement for breast cancer detection, tissue vascular oxygenation, and functional brain imaging. [1]

Though discovered in the nineteenth century, Near Infrared Spectroscopy was introduced to the industry in the 1950s. At first, it was used only as an accompaniment to other optical devices already familiar to the industry such as ultraviolet (UV) and visible (Vis) spectrometers. In the 80s a stand-alone NIRS system was made available, but its application was focused on chemical analysis. At present, Near Infrared Spectroscopy is used as a powerful tool for medical research and in a number of scientific fields including physics, physiology, and as a medical tool for monitoring patients. NIRS is also used in optical tomography (OT) for functional mapping of the human cortex, which is illustrated in **(Figure 1)**.

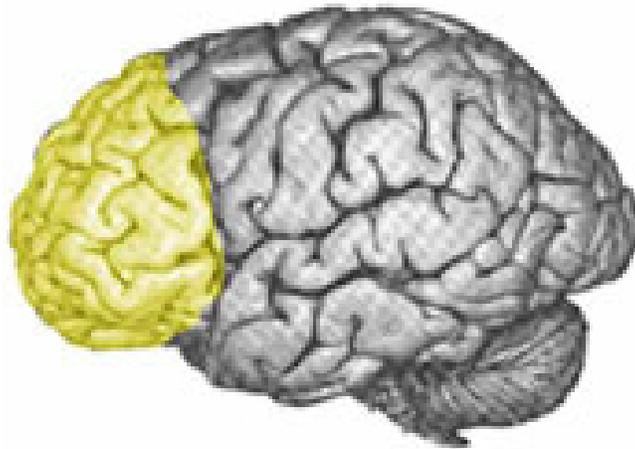


Figure 1: The human brain with the pre-frontal cortex highlighted in yellow. [2]

2.1 Current Methods

Since 1973, which was the year when the first metabolic image of the human brain was produced, technology has been developed to produce better and more ways to produce images. One such method, positron emission tomography or PET scan (**Figure 3**) acquires physiological images based on the detection of radiation from the emission of positrons (tiny particles emitted from a radioactive substance administered to the patient) injected into the skin.



Figure 3: A positron emission tomography (PET) scanner. [3]

Though most commonly used for cancer detection and to examine the effects of cancer therapy by characterizing biochemical changes in the cancer, PET scans can also be used for brain imaging. PET scans of the brain are used to evaluate patients who have memory disorders of an undetermined cause, suspected or proven brain tumors, or seizure disorders that are not responsive to medical therapy and are therefore candidates for surgery. The PET scanner bears a resemblance to a large doughnut and includes multiple rings of detectors that record emission of energy from radioactive substances in the body. The subject is to remain still (immobilization) during the scanning procedure which is about 45 minutes in length. [3]

Electroencephalography (**Figure 4**) measures the electrical activity of the brain by recording from electrodes most commonly on the scalp but in special cases subdurally or in the cerebral cortex. [4]



Figure 4: A patient undergoing EEG.

The results are the electroencephalogram (EEG), which represent an electrical signal from a large number of neurons. It is most useful for classifying epilepsy and syncope, examining sleep disorders, diagnosing comas, and assessing brain death. [4]

Functional Magnetic Resonance Imaging (fMRI) is a technique used to detect subtle increases in blood flow associated with activation of parts of the brain. (**Figure 5**)



Figure 5: Berkeley's 4T fMRI scanner. [5]

It is useful for what is frequently called brain "mapping". [6] It takes advantage of the fact that activation of an area of the brain causes an increase in blood flow to that area that is greater than that needed to keep up with oxygen demands of tissues. This results in a net increase in intravascular oxyhemoglobin (Hb) and a decrease in deoxyhemoglobin (HbO₂). It uses radio waves and a strong magnetic field rather than x-rays to provide clear and detailed pictures of internal organs and tissues. [6] The procedure causes no pain, but similar to PET imaging, the subject must be immobilized. This instrument is also very expensive. The fMRI machine itself could cost between \$1.5 and \$4 million.

2.2 Further Background

It has been suggested that certain cognitive functions induce changes in the blood volume levels of the prefrontal cortex of the brain, and that observing these changes in blood volume levels can be used to predict these same cognitive thought processes. Because of the sensitivity and inability to measure these changes upon stimulation of cognitive function, non-invasive measures are necessary. Biological tissue is transparent to light in the near infrared range between 700-900 nanometers. Therefore, this wavelength range acts as an optical window for the non-invasive assessment of brain tissue. Several NIRS studies in recent years have demonstrated that changes in brain activity can be assessed non-invasively in adult human subjects. [7]

Visually opaque media are omnipresent in nature. While some materials are called opaque because they strongly absorb visible light, others are called opaque because photons traveling within them are largely scattered rather than absorbed. Light is transported through these materials in a process similar to heat diffusion. These so called “diffusing photons” are used in the medical and biophysics communities to view the functions and structure of the body. **(Figure 2)** This is made possible by a spectral window that exists within tissues in the 700-900 nm region, in which photon transport is dominated by scattering rather than absorption. [8]

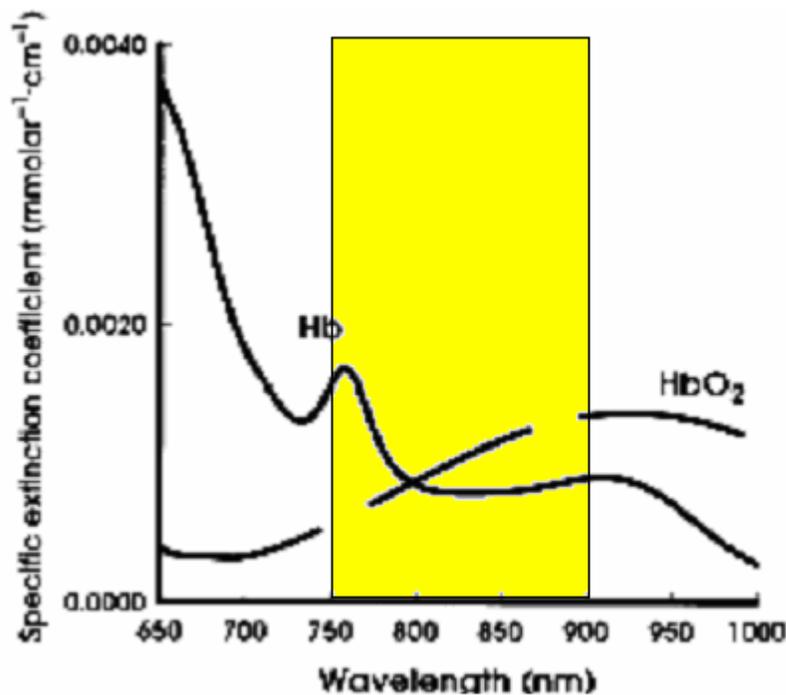


Figure 2: The absorption spectrum of oxyhemoglobin (HbO₂) and

deoxyhemoglobin (Hb) in the near-infrared region 65—1000 nanometers. The highlighted yellow region represents the “spectral window” of 750-900 nm that exists within tissues.

3. Future Uses

Many ground-breaking studies are being executed to explore the use of NIRS in lie detection. NIRS measures changes in Blood oxygenation due to neural activity in the cortex of the brain. Because neural pathways in the brain mediate both the cognitive components of conscious deception, as well as emotional responses NIRS can be used to differentiate intentional deception as well as guilt.

A future use of near infrared spectroscopy as mentioned earlier may be early breast cancer detection. It has been shown that the mammogram does not work well on women under the age of 40. This is partly because women under 40 have more radiographically dense tissue. In addition, many women with a high risk of getting breast cancer may get it before the age of 40. The handheld sensor device under development by Dr. Chance and his team uses NIR penetrating ~3 cm into breast tissue. Moved around the breast in a circular motion, it detects any lumps in the breast. This would benefit many women including those in underserved populations who are less inclined to obtain a mammogram.

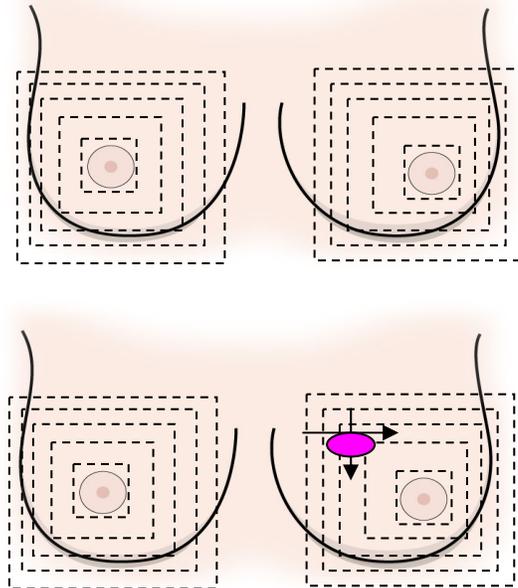


Figure 6: Two example images of results from breast cancer detection exam. The top image is of a normal breast, and the bottom demonstrates where the tumor may be located.

4. Project Goals

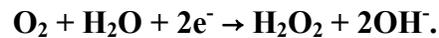
The goal of this project was to use different methods to quantitate oxygen concentration levels in the human brain and correlate these findings near infrared spectroscopy and how it measures these concentration changes in oxyhemoglobin and deoxyhemoglobin. The Cognosensor (brain imager) invented by Dr. Britton Chance, Ph. D, Sc. D, MD was to be used with human blood to represent how Near Infrared Spectroscopy measures oxygen concentration changes in the human brain.

5. Experimental Set-Up

5.1 Oxygen Absorption in Yeast

Introduction

The Instech Clark Oxygen Electrode is a machine that is used to connect with the non-metallic part of a circuit using the equation



This machine could be used to measure the absorption of oxygen in yeast. Yeast was a good starting material because it was cheap, easy to use, and responded to the electrode as the human brain would if the oxygen supply was to be cut off.

Materials

- 1 gram PBS Saline Buffer pH 7.4
- Instech Clarke Oxygen Electrode
- Magnetic Stir Bar
- Magnetic Stirrer
- Salt
- Water
- 7 grams yeast
- 400 ml beaker

Methods

Saline Buffer, salt, and yeast were added to a 400 ml beaker. Water was added to the 100 ml mark. The magnetic stirrer was used to mix the solution. A separate beaker of water was placed next to the beaker. The electrode was placed in the water and set to 100% absorbency reading. Next, the electrode was placed inside of the yeast solution and the time was taken for the reading to reach 0% absorbency. The electrode was again placed in the water and the time was taken for the electrode to reach a stable reading. This procedure was repeated for a duration of seven minutes.

Data

The data was recorded and graphed. (Figure 6)

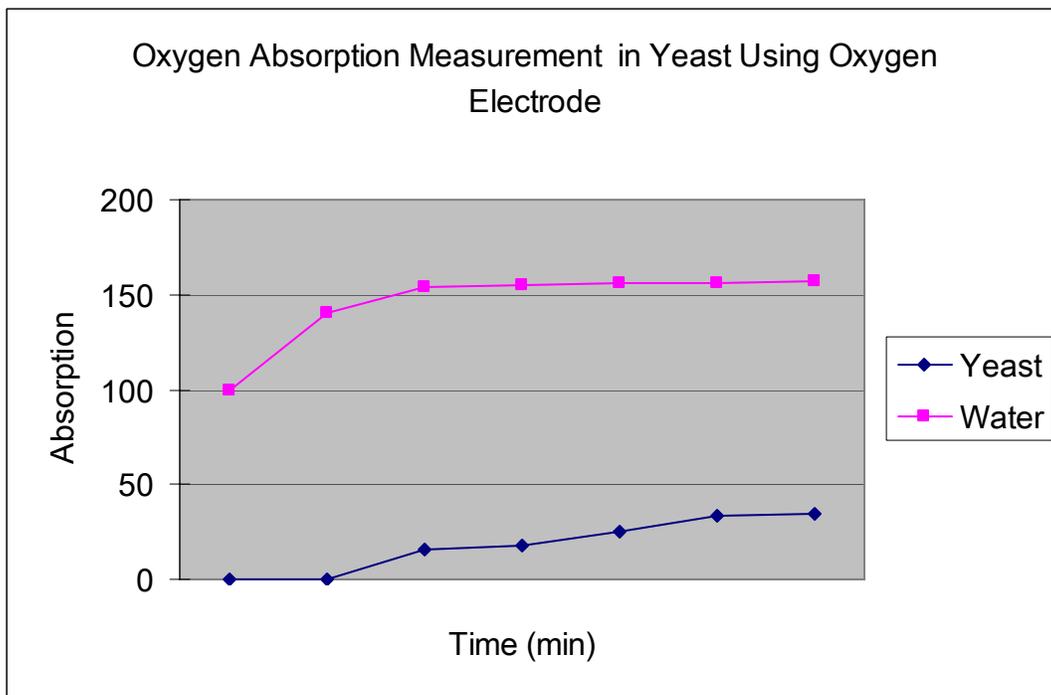


Figure 7: Oxygen Absorption in Yeast Experiment 1. Absorption plotted against time.

Conclusions

As water was introduced into the yeast solution, the percent absorption of oxygen increased.

Discussion

This experiment was conducted many times throughout the ten-week span. The basic purpose of the experiment was to introduce me to the Instech Oxygen Electrode.



Figure 8: Clarke Electrode.

5.2 Measurement of Oxygen Concentration Level Changes in Hemoglobin

Introduction

Materials

- Instech Clarke Oxygen Electrode
- Magnetic Stir Bar
- Magnetic Stirrer
- Water
- Hemoglobin
- PBS Sigma Saline Buffer pH 7.4
- Salt
- Liposyn Intralipid
- Oxygen tank
- Thermometer
- Syringe
- Yeast
- 2000 ml beaker

Methods

The apparatus was set up. Next, 200 ml water was added to the 2000 ml beaker. One package Saline buffer and one gram salt were added. A syringe was used to add 4 ml hemoglobin. The magnetic stirrer was used to mix the solution, a thermometer was used to ensure the solution remained at optimal temperature between 24-25° C. A separate container was used to mix 1.5 g yeast with a sample of the hemoglobin solution, which was then added to the beaker. A syringe was used to add 2 ml Liposyn Intralipid. The Electrode was connected to the solution. The Oxygen tank was connected to the solution and turned on. This introduces oxygen to the solution, and forms oxygenated (oxy) hemoglobin (HbO₂). The beaker was sealed. An initial reading was taken and subsequent readings were taken every minute. After the a stable reading was taken, the Oxygen tank was turned off and a reading was taken every minute until the Electrode read stable absorption level.

Data

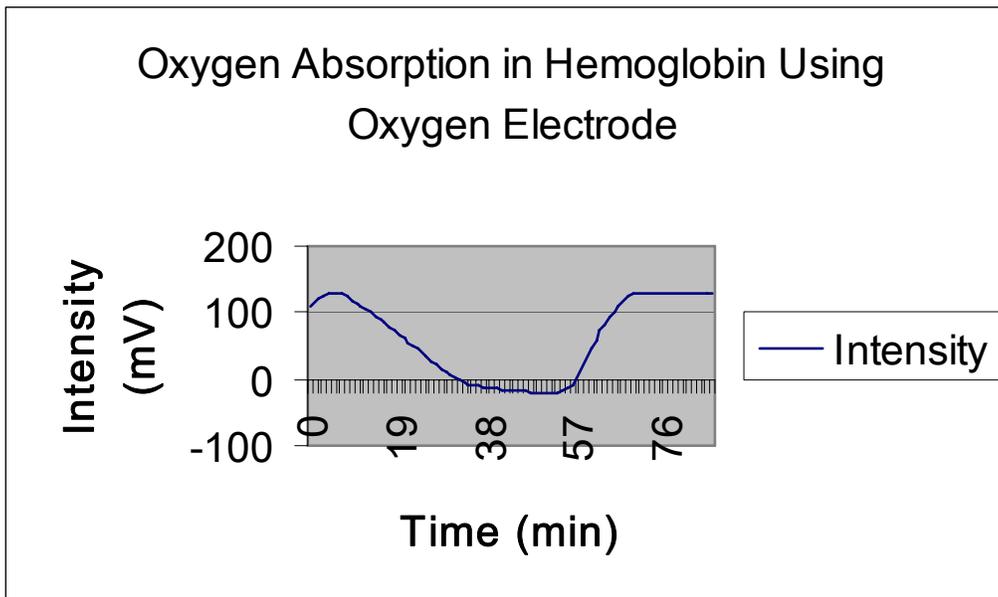


Figure 9: Results graph Oxygen Absorption in Hemoglobin Using Oxygen Electrode

5.3 Measurement of Oxygen Concentration Level Changes Using Both the Clark Oxygen Electrode and the Cognosensor (Brain Imager)

Introduction

Next, I graduated to using the same technique with the Electrode and adding the Cognosensor to study how Near Infrared Spectroscopy detects changes in blood oxygen levels in the human brain.

Materials

- Salt
- Water
- Magnetic Stir Bar
- Magnetic Stirrer
- Thermometer
- Liposyn Intralipid
- Hemoglobin
- Duct tape
- Instech Clarke Oxygen Electrode
- Yeast
- PBS Sigma Saline Buffer
- Hemoglobin
- Oxygen Fluorometer
- 2000 ml beaker

Methods

One thousand milliliters water was added to the beaker. Next, 17 cc Liposyn Intralipid, salt, and 1 pack saline buffer were added. A syringe was used to add 20 ml hemoglobin. The magnetic stirrer was used to mix the solution. The thermometer was used to ensure the solution stayed at optimal temperature of 25-30° C. The electrode was placed inside of and the O₂ tank was connected to the solution. The beaker was sealed. Oxygen was turned on, making oxygenated hemoglobin (HbO₂). Once a stable reading was taken, the tank was turned on and a reading taken every minute until stable.

Data

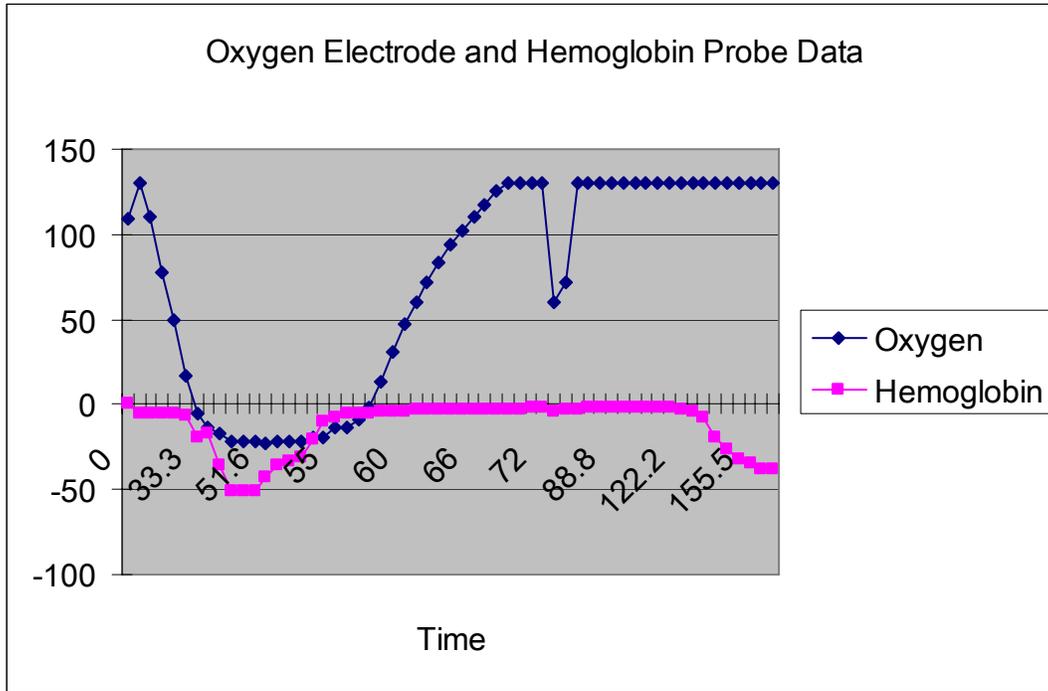


Figure 10: Graphed data from experiment Oxygen Electrode and Hemoglobin Probe Data.

Conclusion

The electrode and brain imager should show increases and decreases in oxygen level concentration level changes at the same time. The results on this graph may be due to systematic error of the electrode.

6. Discussion and Conclusions

Throughout the 10 week research period, there were several dilemmas with equipment, predominately the Clarke Oxygen Electrode. At one point, it became necessary to order another machine. This hindered my research greatly.

7. Recommendations

The next step would be to employ the same technique used on the experiment and to conduct an experiment using the Cognosensor and a rat brain.

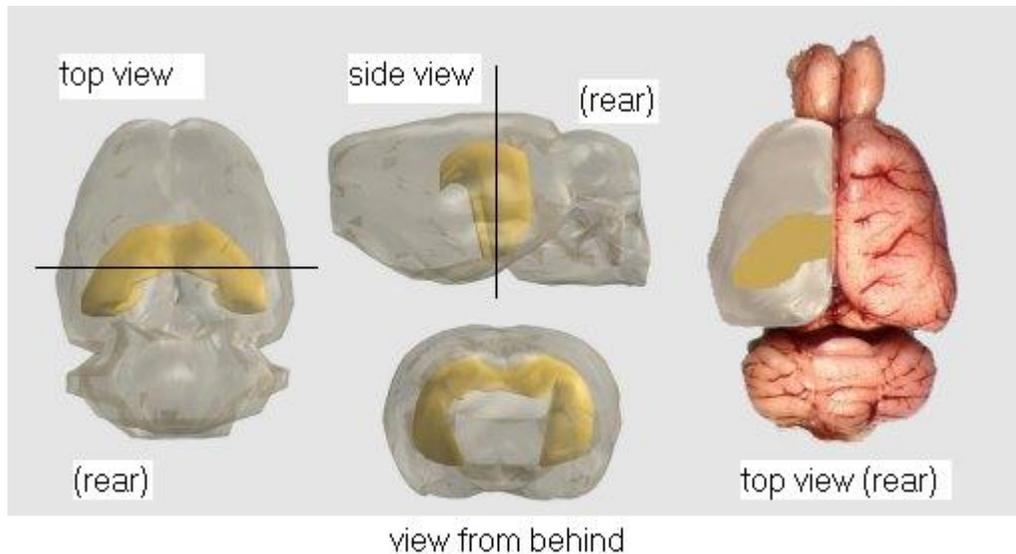


Figure 8: View of the entire rat brain

8. Acknowledgements

I have learned several lessons throughout this summer, including that a chief principal of research is that it is not always as organized as you want it to be. I would like to thank the National Science Foundation for providing and funding this opportunity. This was my first research experience and I can truly say that I am not only a better student, but a stronger person because of it. Thank you, all of my fellow SUNFEST students. Because of you this experience has not only been an educational one, but pleasurable as well. I would like to thank Dr. Jan Van der Spiegel for his kindness, genuine concern for the SUNFEST students and their research, and his dedication to the SUNFEST program. I would like to thank all of Dr. Van der Spiegel's administrative assistants. I would like to thank Ms. Delores Magobet for helpfulness with a few "technicalities" that occurred this summer. Finally, I would like to thank Dr. Britton Chance and his laboratory, especially Juan Du for her supervision and assistance. Dr. Chance has been a blessing this summer.

He was always available for help; he was patient and honestly cared about my SUNFEST experience.

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