UW-MADISON COLLEGE OF ENGINEERING
DEPARTMENT OF BIOMEDICAL ENGINEERING

A device for in vivo 2-photon imaging of synapses in mobile mice

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Background:

The function of sleep remains one of the greatest unsolved mysteries in modern science. A multitude of theories have been proposed regarding the necessity of sleep, but none have been substantively established.

Sleep is known to be an essential process of life, taking up almost 1/3 of the average human's lifetime. Furthermore, it is such a necessity that deprivation has been known to cause health problems in rats, leading up to death (Everson 1995). Additionally, the effects of sleep have been studied on a variety of life factors. Retaining homeostasis is a commonly accepted view, since studies have shown that lack of sleep hampers healthy metabolic activity and immune system response (Zager, 2007). Furthermore, sleep has been linked to proper memory function—lack of sleep has been shown to correlate with cognitive impairment, decreasing by as much as 38% in comparison to a control (Turner, 2007). However, these functions all provide effects of sleep; they do not examine the purpose of it.

A hypothesis has been proposed by Dr. Giulio Tononi that sleep is used for synaptic downsizing. Specifically, he states that “The synaptic homeostasis hypothesis claims that plastic processes during wakefulness result in a net increase in synaptic strength in many brain circuits; during sleep, synaptic strength is globally downscaled to a baseline level that is energetically sustainable and beneficial for memory and performance” (Tononi, 2005). An experiment to test this hypothesis has been proposed using 2-photon microscopy to image synaptic activity in the brain in awake and sleeping mice.

In order to test this hypothesis, our project is centered on the creation of a stereotaxic frame and support system for 2-photon microscopy in mice. This device needs to be able to hold a mouse's head in a fixed position such that the microscope can be used to image neurological activity through a cranial window. Furthermore, the mouse must be as free of stress as possible, must be supported from underneath, and given free movement.

Design Problem:

In order to successfully test the hypothesis of synaptic downscaling as a neurological function of sleep, a device capable of holding a mouse's head in a fixed position for 2-photon microscopy is necessary. The device should be broken into two parts—a stereotaxic frame for keeping the mouse's head in a rigid position, and a stage upon which the mouse can have freedom of movement.

Client Requirements:

The clients, Dr. Giulio Tononi and Dr. Ugo Faraguna would like our team to develop and construct a device for use with a 2-photon microscope. The device is made up of two modules: a frame that holds the mouse's skull in a fixed position for microscopy of the cranial window, and a "treadmill" that allows freedom of movement for the mouse. The client would like the treadmill to be done as quickly as possible for design purposes and for training the animals. Important considerations include the fact that the device must have no electrical components; the treadmill should ideally provide no movement restrictions; and that the device must fit between the lens of the microscope and the table on which the microscope rests.

Similar Products:

Researchers at Princeton University have developed a similar frame for use with a 2-photon microscope (Fig. 1).

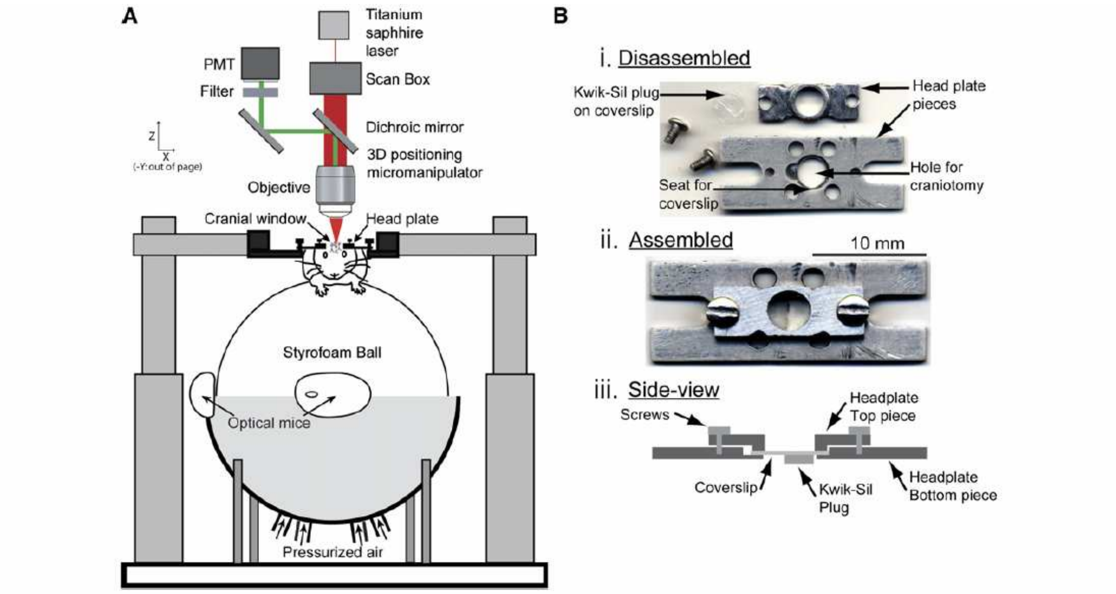


Figure 1. Two-Photon Microscopy Experimental Apparatus for Awake Mouse Imaging
(A) A two-photon microscope is used to image through the cranial window of an awake, behaving mouse that is able to maneuver on an air-supported free-floating Styrofoam ball that acts as a spherical treadmill. Optical computer mice are used to record mouse locomotion by quantifying treadmill movement.
(B) Images of the disassembled (Bi) and assembled (top-view [Bii] and side-view [Biii]) head plate used for cranial window imaging and mouse head restraint.

(Image from Dombeck, 2007)

The device is designed for use primarily with awake, mobile mice. Of particular note is the use of optical computer tracking mice for recording the specific movement and speed of the ball upon which the mouse rests. Additionally, the use of pressurized air to lift the ball and create an extremely low-friction environment allows for examination of brain activity solely for locomotion, avoiding potential issues with using the head restraint as something with which to exert leverage to move the ball.

Product Uniqueness:

Our design will be superficially similar to the one used by the Princeton researchers. For the head restraint we will be using replaceable and ideally “snap-together” plastic pieces for easy placement of the mouse in the frame. Furthermore, we will not be using pressurized air as a method of letting the ball rotate, opting instead for ball bearings mainly for economical purposes.

Design Alternatives for treadmill:

In order to provide a device allowing for freedom of movement in all directions to a mouse of size <50g, limiting resistance was the major priority in the design process. However, to complicate the issue, the design also needed to incorporate enough friction for the mouse to easily grip and run on the device. The design also needed to provide enough durability to withstand multiple experiments, lasting approximately 24 hours each, easily cleaned since the mice would likely urinate on the device, and adjustable to account for variation of the mice and different microscope objectives. Compatibility with the microscope was critical, the device would need to fit beneath the 2-photon microscope, which has a clearance of approximately 8.5" and not damage any of the equipment when in use. The device should also be quiet during operation and comfortable for the mice in order to provide an appropriate environment to allow the mice to sleep naturally.

Water Suspension:

The water suspension device is quite simple in design and ideal in reducing frictional forces, providing a fluid interface in which a ball could easily move in 2-dimensions. The basic concept would consist of a small box with a hole cut in the center, tapered downward in a cone shape, and filled with water or equivalent fluid. This would fix the ball in one position and prevent it from floating away from the restrained mouse. Also, this would limit the possibility for water to splash out and ruin the electrical components of the microscope. The device could be constructed from Plexiglas, making it relatively cheap and easy to construct. Since the ball would be in contact with the water, it would be constantly cleaned, but would require replacement of the water after each experiment. This would also mean the ball would always be wet, which could be hazardous for the mouse since it would be difficult to grip, allowing for the possibility of slipping and injury. Also, due to the long-term use of the device, it is possible a leak in the box design could develop or water could be spilled while cleaning the device, destroying $1.2 million worth of equipment. For these reasons, it was concluded the water suspension would not be the best solution to this design problem.

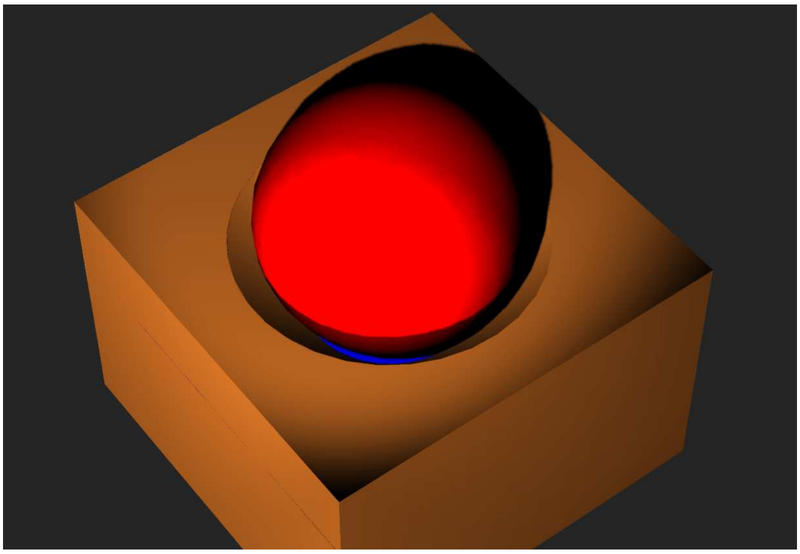
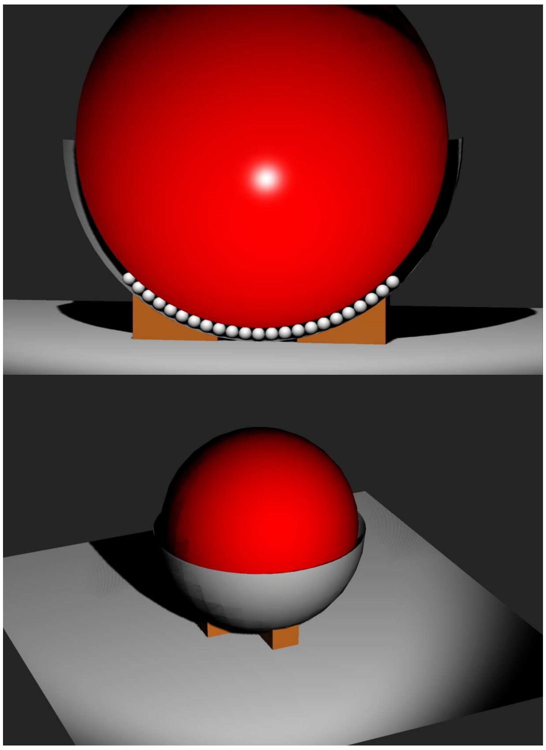


Figure 2. Water Suspension Device. This image shows how a ball could be suspended in water held in a box in a way that holds its position and limits splashing.

Ball Bearing in Bowl:

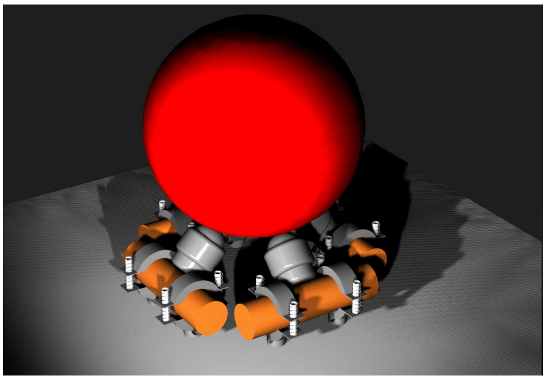
The second design alternative was to use small ball bearing in a metal bowl and place a larger ball on top as seen in Figures 3 and 4. This keeps the ball in a fixed position, but at the same time allows for 2-dimensional movement. However, due to multiple contact sites on the metal ball bearings, the frictional forces would likely be much greater than the water suspension design, but if the mouse is strong enough, this device may be feasible. Also, this design would be difficult to clean, since all the ball bearings would get dirty if the mouse urinates. The device would be relatively simple to build, but since 50-100 ball bearings and a metal sphere would be needed, the costs for would be much higher than the other designs. The mouse is likely more compatible with this design because the ball would remain dry and allow for greater traction. However, this traction compromises the resistance of the design and the multiple contact sites with the ball bearings give this design the highest frictional forces of the three proposed design alternatives.

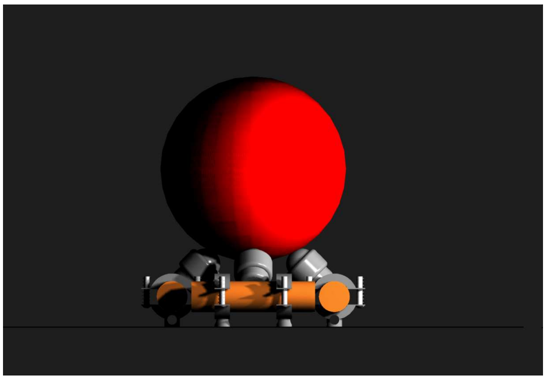


Figures 3 & 4. Ball Bearing in Bowl device perspective and side views.

Four Large Ball Bearings:

The final design alternative is based on a modification of the previous ball bearing in bowl design as seen in Figures 5 and 6. This design also allows for 2-dimensional movement, however, only 4 ball bearings are used, reducing the resistance involved in rotating the ball. The ball bearings are connected to dowels and held in place using electrical clamps. The angle of these ball bearings can be adjusted so all four bearings touch the ball exactly, evenly distributing the load and allowing for smooth motion. This alternative also makes the device easier to clean and more durable since the ball bearings are not rubbing against each other as in the previous design. However, using the ball bearings increases the frictional forces involved in rotating the ball in comparison with the water design, but it is believed the mouse will be strong enough to overcome this force. This design would also be relative easy to build and inexpensive.





Figures 5 & 6. Four Large Ball Bearing device perspective and side views.

Design Matrix:

After analyzing the pros and cons of the previous three designs, the following design matrix was created, which takes into account the ability of the design to allow for 2-dimensional motion, frictional forces, cost, ease of operation and cleaning, mouse compatibility, durability, build difficulty, and microscope compatibility. It was concluded that the four large ball bearing design would be the best solution in solving this design problem.

	Four Large Bearings	Bearings in Bowl	Water Suspension
2D movement (5)	5	5	5
Friction/Resistance (20)	15	10	20
Cost (5)	5	2	4
Ease of Operation/Cleaning (10)	8	4	7
Mouse Compatibility (15)	15	15	10
Durability (20)	17	15	17
Build difficulty (5)	2	5	3
Microscope Compatiblity (15)	15	15	10
Adjustability (5)	5	1	3
TOTAL	**87**	**72**	**79**

Testing:

The testing for our design was done in two phases. The first phase focused on testing the “ball treadmill” while the second phase focused on testing of the head restraint and frame. For ease of reading, the “ball treadmill” phase will be referred to as phase 1, while the head restraint and frame will be referred to as phase 2. Phase 1 testing took place in two initial designs: the box design and the flat design. To test both these designs, the ball was placed on the bearings in their respective set ups and rolled by hand with varying degrees of force.

Phase 1:

Box Design:

The box design testing revealed that the bearings touching the ball must be adjustable. Our team realized that our tools did not have enough precision in order to make sure all the bearings were aligned correctly. This was evident when the ball was observed to balance on only two or three of the bearings at one time, rather than on all four.

Flat Design:

The flat design showed that if the ball was in contact with all four of the bearings it would roll smoothly. After rolling the ball with substantial force, it was evident that in this design, the ball was not able to stay on the bearings at a high rolling rate and therefore would not be sufficient for our final design.

Testing Box Design with Untrained Mice:

After testing both the flat and boxed design, our team had a meeting with the client. The client viewed both of the aforementioned prototypes and thought it would be advantageous to test at least one of the design ideas to give an idea of how the mouse would react to the bearings and ball. The design idea in the most shape to be tested was the box design. During the test, the mouse was held in place by the tail and placed on the ball. The mouse was able to run, but it is important to note that the mouse was not properly trained to run on the ball at this time.

It was observed that the mouse had issues with the slipperiness of the surface of the ball. The mouse became quickly agitated and urinated on the ball. This presented two new challenges for our team. First, we had to make sure the ball could be washed (waterproof), and secondly the surface of ball would need adjustments in order for the mouse to be able to grip it.

Prototype Design:

Due to the short amount of time required from our client to build our prototype, it was imperative that the group decide on a prototype design as fast as possible. After completing the necessary "blue sky" phase and testing these designs at the lab, a final design was quickly chosen using the design matrix shown in the design alternatives section. As previously mentioned, the "4 bearing" idea was the prototype chosen by the group. As mentioned above in testing, there were two main designs within the category of the "4 bearing" design. Testing these designs helped us refine the flat design and the box design into one efficient prototype. The following section will explain each portion of the final design, and how the problems found in testing were fixed to help create the final prototype. To see pictures of the final prototype, refer to Appendix C.

Ball:

The Ball Itself:

The ball itself is arguably the most important part of the design given that it is the connection between the mouse and the bearings. After searching for hours in countless stores across town, the group stumbled across an unbreakable, enlarged Christmas ornament. This ornament was the perfect size approximately 6 inches in diameter, and was composed of an interesting deformable plastic that we thought would be sufficient for the mouse run on (note even though the plastic was slightly deformable, it was hard enough to not deform with contact from the bearings). Even though the ornament had a notch protruding from the side to hang on a Christmas tree, it was easily filed off and filled over with multiple layers of tape.

Mesh on the Ball:

In order for the mouse to be able to run, the ball needed to possess proper traction on the surface. As mentioned in the testing section, it was evident in our first design the used; drywall mesh. Drywall mesh, see figure(7), has adhesive on one side while having no adhesive on the opposite side. It also, as seen in the name, has a mesh like design, giving the mice adequate spaces spanning the entire surface of the ball in which to grip. In order to put the mesh on the ball, cuts were made in the mesh to make sure there was no protruding “mesh bubbles” on the ball. To make sure the mesh would stick, waterproofing spray was used to not only waterproof the ball, (talked about in next paragraph) but to also seal the mesh on the ball itself.

Figure 7: Drywall mesh used on the ball.

At first, it appeared that the mesh would cause the ball to roll with more friction on the bearings because of the "dimply" appearance of the mesh. Fortunately, the mesh did not cause a problem in terms of ease of rolling on the bearings. The group hypothesizes that this is due to the fact that the dimples in the mesh are an order or so smaller than the size of the bearings, although this still needs to be measured quantitatively.

Waterproofing the Ball:

After finishing the mesh portion of the ball, not only did we need to coat the ball in a substance that would help the mesh stick, but it was also imperative for the ball to be waterproof. In order to waterproof the ball, two sealants were purchased; a light waterproof spray on sealant called clear Rust-Oleum, as well as a rubber spray meant to be used on the bottom of a car. Both sprays were tested on test balls. It was obvious that the rubber spray would be hard to apply so that the ball was covered uniformly, even though it, in theory, be easier for the mouse to run on. For these reasons, the group decided to use the Rust-Oleum. Before the sealant was applied to the ball, it was applied to the mesh to make sure it would properly hold the mesh to the ball, which it proved to do. Because the waterproof sealant was so fine, we were able to spray multiple layers onto the ball to make sure there were no spots missed. After the spray was completely dry, the ball was placed in water for 10 minutes to make sure it was entirely waterproof, which it also proved to be. This concluded the groups work on the ball.

The Base and Bearings:

Base:

During the testing of the box design with the untrained mouse, it was apparent that wood would not suffice as a bottom base because of cleaning issues. Because of this reason, a 12 inch by 12 inch piece of sheet metal was used as the base. This size was chosen to allow room for the frame as well as to allow for adjustability with respect to where the bearings sit on the ball. As we continued to run tests with sheet metal as our base, it was evident that it was simply not sturdy enough to hold the bearings in place. For this reason, a 12 inch by 12 inch by ¼ inch wood board was added underneath to insure the sheet metal not bend back and forth.

Adjustable Bearings:

As was mentioned in the testing section, the key to the final prototype design was adjustability in the bearings. This was needed to make up for human errors. In order to do this, a few of the following components were needed:

A. Dowels:

To make the bearings 100 percent adjustable (able to move to any range of degree), a dowel was cut to two inches and marked in the middle. The mark in the middle was then drilled out with ¼ inch drill bit and the bearing was placed into the hole. This allowed the bearing to be housed in a sturdy device that had the possibility of rotating. In order to allow rotation while simultaneously being able to lock the dowel and bearing in place, the proper holding device was essential to the success of the project.

B. Electrical Clamps:

After searching in Home Depot for approximately an hour, the group stumbled upon the perfect solution. Eight ground wire electrical clamps were purchased figure(8). These electrical clamps allowed the user to be able to unclamp the dowel with the bearings, rotate the dowel to get the bearing in the proper position, and finally clamp the dowel down to hold the bearing in the proper position.

Figure 8: Electrical ground clamps used in design. Part with one screw is referred to as the top.

Combing Bearings, Ball, and Base:

Now that all the pieces seemed to be properly manufactured, they needed to be placed on the base. Because the number one priority was to get the ball to move, the electrical clamps needed to be placed in a precise position on the base. To do this, the following diagram was used to calculate the correct distances between the bearings.

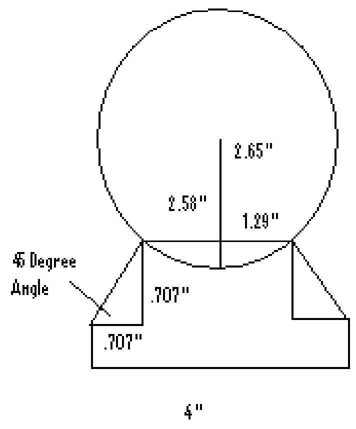


Figure 9: Diagram to calculate distances between bearings.

Using the diagram above we found the distance between the bearings to be four inches. When the position of the bearings was marked properly, holes were drilled for the electrical clamps. These holes were marked 1 inch on both sides of each bearing. After the holes were drilled, the clamps were screwed in so that they were upside down. This may sound strange, but in order for the clamps to fit properly on the base it was imperative that they be screwed in upside down.

When the electrical clamps were successfully screwed into the base, the bearings were adjusted so that the ball barely touched all 4 bearings at the same time. This took a few adjustments but eventually everything worked according to plan. The ball was placed on top of the bearings and was able to roll smoothly without falling off of the bearings.

Future Work:

The next steps our team will take will be to test the ball using trained mice to see if any unforeseen issues arise. Once this is done, we will move on to phase 2, the head restraint and frame. At the moment our team is still brainstorming ideas for the head restraint and frame as well as testing the compatibility of various materials with dental cement. The dental cement is key to the success of phase 2, and based off of the results in our testing, we will experiment with design ideas and later move on the final prototype.

APPENDIX A

Project Design Specifications

PRODUCT DESIGN SPECIFICATIONS

September 15, 2008

Project Title: Device for *in-vivo* 2-photon imaging of synapses in mobile mice

Team:
David Leinweber – Leader
Jon Seaton – Communicator
Mark Reagan – BSAC
Jay Sekhon – BWIG

Function:

2-photon microscopy is a highly useful tool for examining a variety of characteristics. In neuroscience, it has become particularly useful for imaging of synapses in the brain to determine brain function. Other groups have successfully used a cranial window in mice to perform synapse imaging by holding the mouse's skull rigidly to the stage of the microscope while leaving the body free to move. It is the goal of this project to create a similar device that will allow a mouse's head to be held in a fixed stereotaxic frame to be used for examination of synapses during sleep and waking periods.

Client Requirements:

The clients, Dr. Giulio Tononi and Dr. Ugo Faraguna, would like the construction of a fixed stereotaxic frame for 2-photon microscopy. The device is made up of two modules: a frame that holds the mouse's skull in a fixed position for microscopy of the cranial window, and a "treadmill" that allows freedom of movement for the mouse. The client would like the treadmill to be done as quickly as possible for design purposes and for training the animals. Important considerations include the fact that the device must have no electrical components; the treadmill should ideally provide no movement restrictions; and that the device must fit between the lens of the microscope and the table on which the microscope rests.

Design Requirements:

A. The "treadmill"

1. Physical and Operational Characteristics

    a. *Performance Requirements*

    The device should allow at minimum 1-dimensional mobility for the mouse. It should ideally allow complete freedom of movement for the mouse to run, stop, stand, and fall asleep with no discomfort whatsoever.

    b. *Design Restrictions*

    The device must be large enough to support mice of various sizes but also small enough to fit underneath the lens of the microscope and the table on which the microscope rests. No electrical components may be used.

    c. *Materials/Durability*

    Materials used should be inexpensive, but durable and able to withstand long periods of extensive use. The device should require as little maintenance as possible.

2. Production Characteristics

    a. *Time*

    The device should be prototyped and tested as quickly as possible to allow for the mice to be trained properly.

b. *Quantity*

Only one prototype should be necessary provided it meets the functional requirements.

B. The stereotaxic frame

1. Physical and Operational Characteristics

a. *Performance Requirements*

The device should allow for complete immobilization of the head for effective, repeated use of 2-photon microscopy. The immobilization should be constant—that is, when a mouse is held in the stereotaxic frame and then released, when it is placed back in the frame it should be in the same position. The device must have two parts: one attached to the mouse's skull via dental cement and an appropriate attachment point on the frame itself. The frame should have a window that the cranial hole can be seen through. The frame should also have the potential for EEG monitoring.

b. *Design Restrictions*

The frame should be solid enough to prevent movement from the mouse. It should allow for repeated attachment and detachment of the mouse from the microscope stage. The attachment on the mouse's skull should be light and compact to limit restrictions on the mouse's normal mobility. There should be no electrical components aside from the interface to provide EEG monitoring, should that be necessary.

c. *Materials/Durability*

The frame should be extremely durable and able to withstand extensive use for extended periods of time. The attachment to the mouse's skull should ideally be made from plastic and either reusable or cheaply and easily purchased or constructed.

2. Production Characteristics

a. *Time*

The device should be prototyped after the arrival of the microscope to allow for proper measurements.

b. *Quantity*

Only one prototype should be necessary.

APPENDIX B

Expenses

List of Expenses

Wisconsin Craft Market

9/26/2008: Graphite Lube: 1.89 dollars

9/27/2008: 2 wood boards, 4 dowels, 1 paper ball: 20.67 dollars

Ace Hardware

9/26/2008: 5 washers, 5 lock nuts, 5 nuts: 4.22 dollars

Menards

9/26/2008: Jumbo Ornament: 3.15 dollars

Home Depot

10/03/2008: 150 mm ornament, undercoat rubber coating, clear sealant: 12.74 dollars

10/03/2008: Sheet Metal, 8 ground clamps: 18.48 dollars

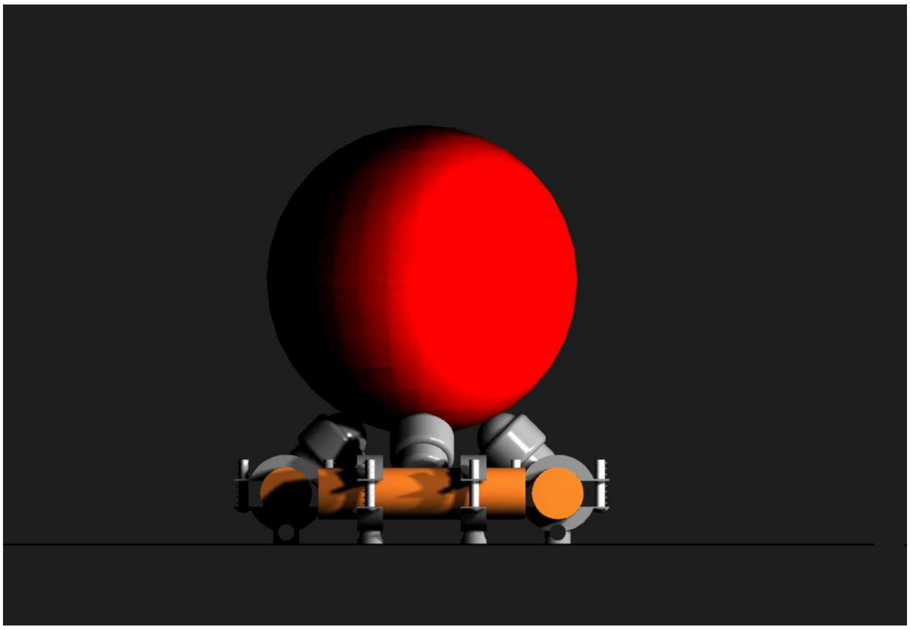
VXB Bearings

9/17/2008: Ceramic Bearings, Housed Bearings: 90.99 dollars

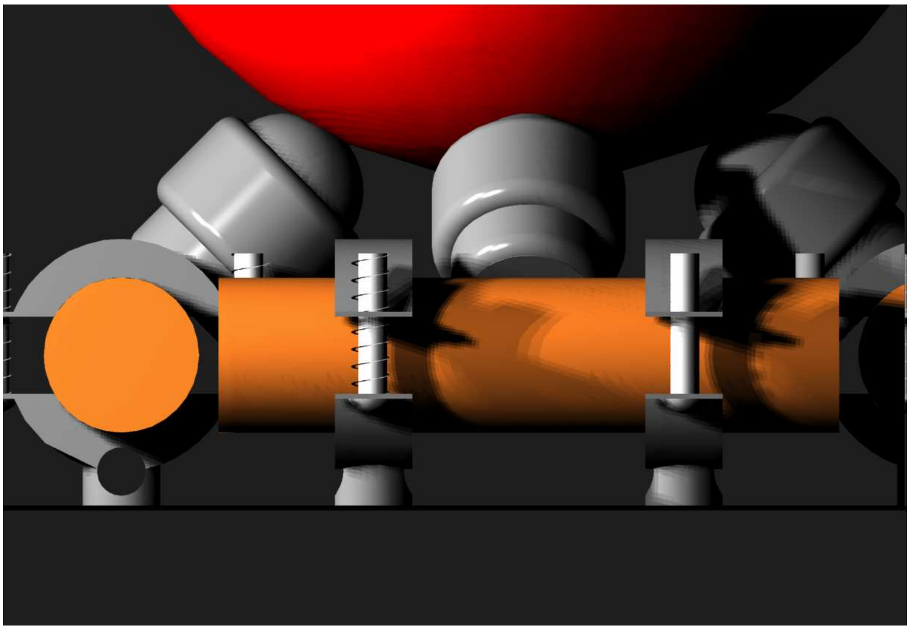
Total: 152.14 dollars

APPENDIX C

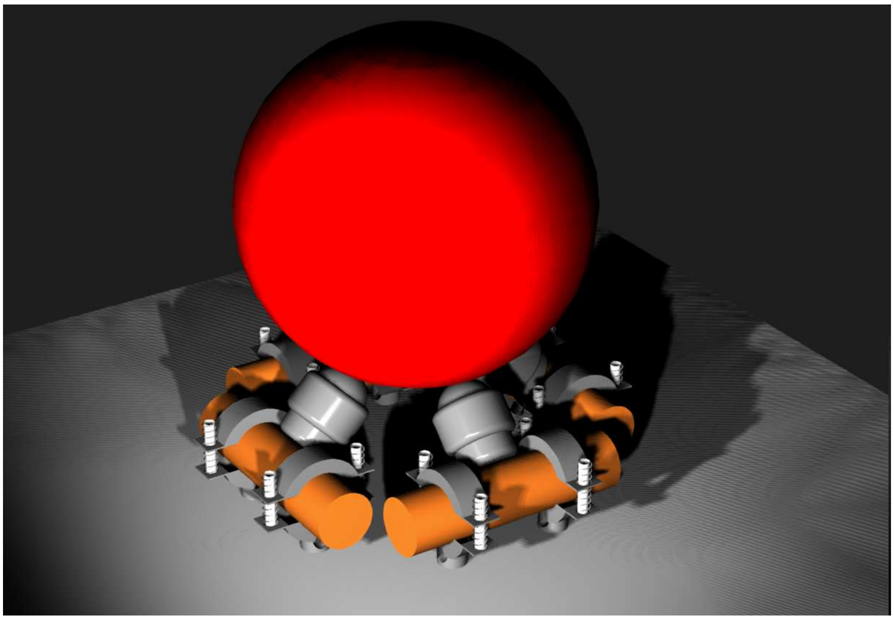
Pictures of Final Design Prototype



Global view of final design



Close up of bearings in dowels and electrical clamps holding dowels in place.



Birds eye view of prototype design. Note how the electrical clamps are put in upside down.

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