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 even 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.

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.

Design Problem:

In order to support 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) A stage upon which the mouse can have freedom of movement. (Phase 1)

B) A stereotaxic frame for keeping the mouse's head in a rigid position. (Phase 2)

Similar Devices:

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

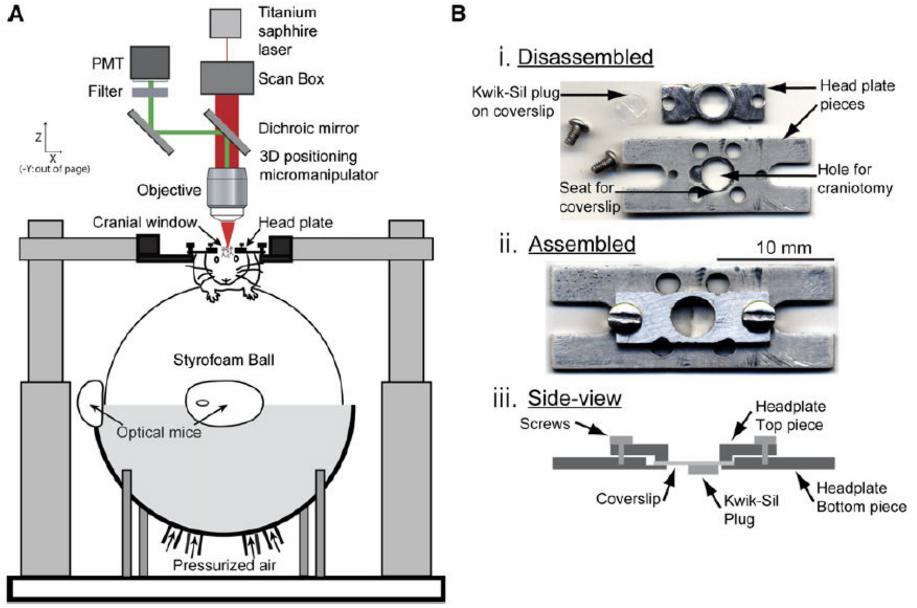


Figure 1. Two-Photon Microscopy Experimental Apparatus for Awake Mouse Imaging

(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 economic reasons.

Phase 1: "Treadmill" Design

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 traction for the mouse to easily grip, as well as low inertia to allow the mouse to run on the device. The design also needed to be durable enough to withstand multiple experiments, lasting approximately 24 hours each, easily cleaned since the mice would likely urinate/defecate on the device (talked about later in testing), and be adjustable to account for variation of the mice and different microscope objectives. Compatibility with the microscope was critical meaning the device would need to fit beneath the 2-photon microscope, which has a clearance of between 8-9 inches 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.

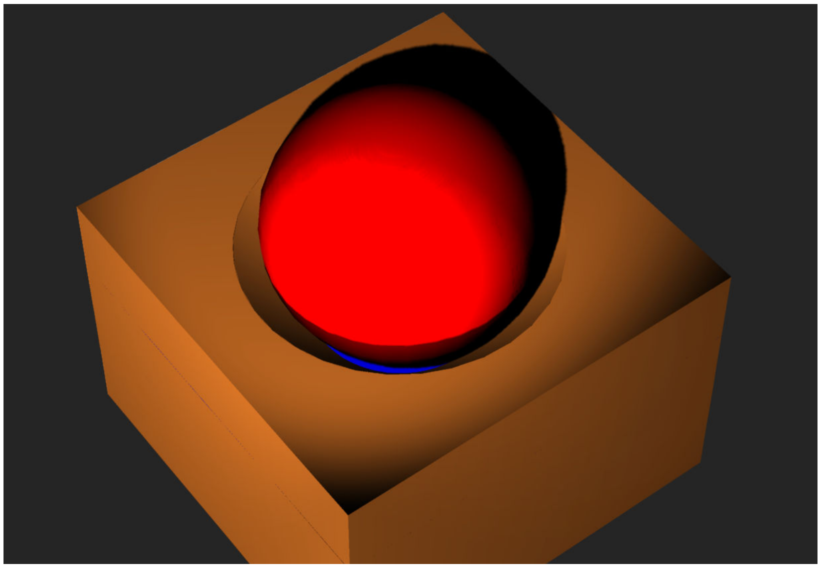
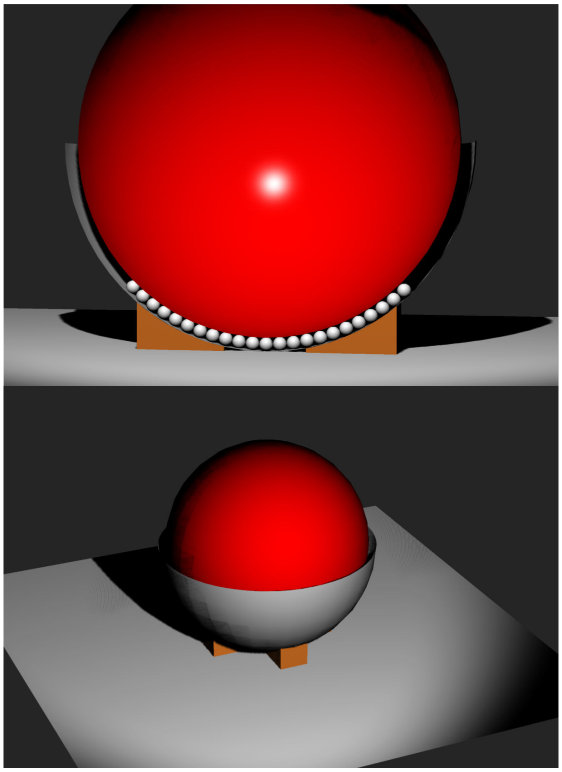


Figure 2. Water Suspension Device. This image shows how a ball could be suspended in water held in a box in a way that holds its position and limits splashing.

Ball Bearing in Bowl:

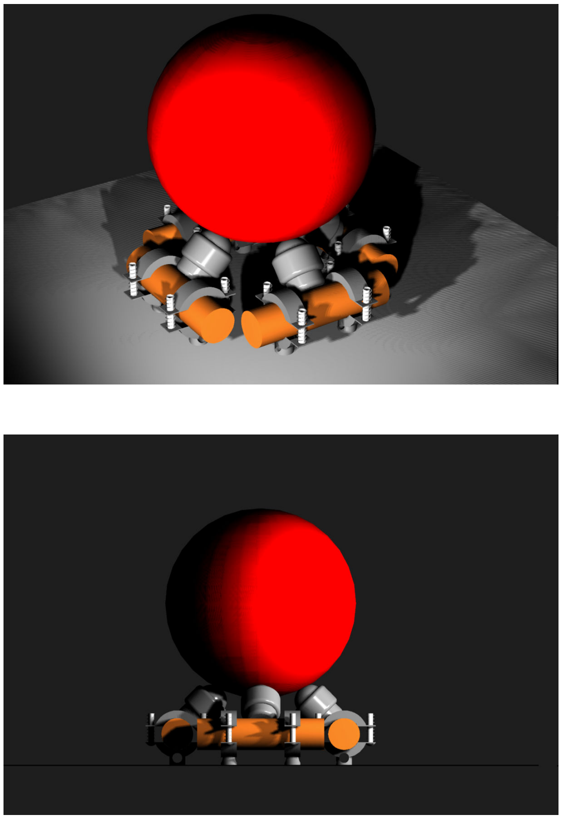
The second design alternative was to use small ball bearing in a metal bowl and place a larger ball on top as seen in Figures 3 and 4. This keeps the ball in a fixed position, but at the same time allows for 2-dimensional movement. However, due to multiple contact sites on the metal ball bearings, the 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 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, 4 ball bearings are used, reducing the resistance involved in rotating the ball. Originally, instead of 4 ball bearings, 3 ball bearings were arranged in a triangle shape. After rolling the ball on the 3 bearing design, it was evident one more bearing was needed for added support, due to the ball falling off of the bearings. 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**

First Final Prototype Design:

*Note: The first prototype design was the prototype built at mid-semester and used by the client for 1 test. This was not the final prototype used. The final prototype used is described in second final 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 first final design, and how the problems found in testing (see testing section) 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. The ball itself is an enlarged Christmas ornament. This ornament an ideal size of approximately 6 inches in diameter, and was composed of an unknown deformable plastic that we thought would be sufficient for the mouse to 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 coated on the ball (talked about in next paragraph).

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 area of the dimples in the mesh are approximately 20 times smaller than the area of the bearings.

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. The ball was sprayed multiple times 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:

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.

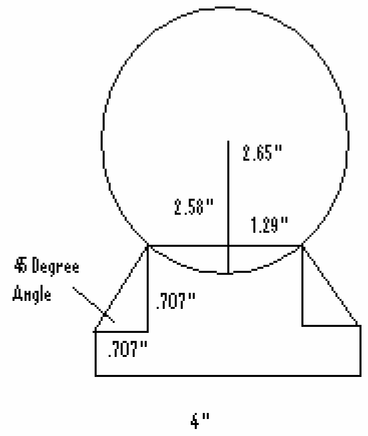


Figure 9: Diagram to calculate distances between bearings.

Knowing that we wanted the ball to rest on the ball bearings about .25 inches from the bottom of the ball, the distance between the bearings was found to be four inches. To find the distance of 4 inches between the bearings, we were able to use simple geometry calculations to find the numbers in the diagram above. 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. In order for the clamps to fit properly on the base they were screwed in upside down due to the design of the clamps.

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.

Testing:

To test the efficiency of rough prototypes constructed in phase one, two different designs were tested: 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 for a rough approximation of a mouse.

Box Design:

Before testing of the box design is talked about, it is necessary to describe the box design. The box design consisted of 4 pieces of wood, each 2 inches wide and 4 inches in length. Each piece of wood was then connected to each other in a square so that the length of the wooden pieces was parallel with the ground. The ball bearings were placed in the middle (1 inch up from the ground, and 2 inches horizontally) from the end of each wood piece. 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:

It is also necessary to describe the flat design before we talk about testing. The flat design consisted of a piece of wood flush with the ground. Four ball bearings were placed vertically in the wood base so each was 2 inches apart. 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 would have to be slightly tweaked for the 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 understand how the mouse would react to the bearings and ball. The design idea that was 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 slipped on the ball as it ran, because it did not have enough traction on 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 to be altered in order for the mouse to be able to grip it.

Second Final Design

*Note: This is the design given to the client at the end of the semester. It is the design currently being used by the client.

After testing the final prototype described above we found the mouse to have more of a challenge moving the ball than the client desired. In order to generate the ideal amount of resistance for the mouse, the client suggested using a 1 dimensional system instead of a two dimensional system of rotation. Basically this meant an axle of some kind instead of a bearing idea. As well as making it easier to have less resistance, the client believed an ‘axle’ design would fit between the stage and the objective of the microscope more efficiently. As well as creating a one dimensional “treadmill”, the client also asked that the treadmill device be adjustable in the z-plane. At first, the client believed this adjustment was not needed. After using the microscope over a span of a few weeks, he realized the adjustability in the z-plane was indeed necessary. In order to meet the new design criteria, a complete overhaul of the existing design was necessary. The following section will describe the final prototype and all of its components based on the picture with labels below.

Component A) Linear Actuator: The linear actuator is essential for moving the device up and down. Initially, a slider was built out of a garage door slider (see appendix G). This device worked, but was inefficient and was hard to adjust with your hands when placed in the microscope stage. Because it seemed very difficult to build a device that would be efficient by hand before the deadline, research was done to find out if a motorized slider device was sold on the market. The team purchased a linear actuator track that was identical to the previous slider device, but was adjustable via motor and allowed for fine adjustments.

The linear actuator track measures 11 inches tall, creating a potential size problem as the slider needs to fit between the stage and the objective of the microscope. Fortunately, after taking measurements on the stage that holds the microscope, we found sufficient space to place the linear actuator track. In order to move the actual treadmill (the ball) up and down, the component that holds the rod to act as an axle for the ball, must be attached to the linear actuator. Using the screw holes that came stock with the linear actuator track, the ball bearings were screwed in place. It was essential that these ball bearings be extremely rigid so the rod does not wobble. For this we used ¼ inch bolts to secure the ball bearings to the plate on the linear actuator track.

Component B) Axle and Treadmill Ball. The two aforementioned components are arguably the most important parts of the device, yet probably the most simple. In order to create the Axle-Ball device, the ball needed to be drilled directly in the center with a ¼ bit to allow the Axle to fit through snuggly. In order to drill a hole perfectly in the center, the ball was placed in a hole cut in a circle and clamped. After the bit was aligned perfectly in the center using a fishing line with a weight on the bottom, the hole was drilled. The axle, a 15 inch steel rod, was placed through the middle of the ball. The Axle-Ball device was now complete. The axle was placed inside the bearings located on the linear actuator track. At this point the device, in theory, could be used using support of the axle from only one side.

In order to test if the device would work with support from one side, a mouse was held by its tail and put on the ball while measuring the length the axle moved from the center of the bearing. If there was too much play ~.1 cm, another holding device would be needed in order to keep the axle and ball device more steady. After measuring the play with a meter stick and taking the average of 3 trials, we found the average play to be ~.3 cm. Because this number was higher than .1, it was decided that another support on the side opposite the linear actuator was necessary.

Component C) Opposite side support. Because it was found there was significant play in the axle with support from only one side, a support on the opposite side of the linear actuator track was necessary. This support is similar to the slide built to be replaced by the linear actuator track. Just like on the linear actuator track, a ball bearing device was connected to a plate that can slide up and down in a garage slider. The ball bearing can be locked down in place by a aluminum stopper that connects to the garage slider. In order to adjust this support, one must manually change its position. Unfortunately a manually operated slide in any part of the design was not ideal. In the future, it may be smart to replace this device with springs.

Phase 2: Head Restraint

Design Alternatives for the Head Restraint

In order to provide a device capable of fixating a mouse and imaging at the micron scale, the primary criterion for the head restraint was rigidity. It was important to construct the device out of a material that would not compress under the stress from the mouse otherwise the imagining of the individual neurons would be inconsistent. Another important factor in the design process was the ability for the restraint to be adjustable in the z-axis to fit different objectives. The microscope stage itself was equipped with fine z-axis adjustment, however our device needed to incorporate a course ½" adjustment. The device would also need to be easy to operate, fixing and removing the mice, cleanable, and compatible with the 2-photon microscope. Aside from the above, the device should also be relatively easy to build and cost effective.

Wood restraint

In this design, the mouse would be fixed using small neodymium magnets attached to a small wooden dowel spanning a 12” gap. This dowel would have two wooden support plates on either side with holes drilled to allow for adjustability. Adjusting the height of the device would involve sliding the dowel out of its hole and moving it to an alternative position. The holes would be drilled in a way to allow for a snug fit to prevent unwanted movement. Some benefits of this design would be ease of construction and cost. However, the problems with a device such as this would be the flexibility of the wooden dowel and the ability to be cleaned. Being made of wood makes it difficult to wash after use, which in turn could pose a heath hazard as bacteria and other materials may become imbedded in the wood. Also, since wood can easily be compressed, the device would allow for undesirable movements. It may also be difficult to adjust the height of this device due to the snug fit of the dowel in the wooden holes, forcing the operator to tug on the device potentially leading to failure.

K’nex Restraint

In the K’nex design, the mouse would be fixed by cementing a small K’nex piece during surgery that would later clip in to a K’nex bar located just above the microscope objective. The bar would be fixed on each end in a way in which it could easily be “snapped” in and out and adjusted with the necessary increments. Some major benefits of this design are its ease of use, cost, and ease of constructions. However, since the spanning gap between the ends of K’nex bar would be approximately 12”, the acrylonitrile butadiene styrene (ABS) plastic in which K'nex are made would be less rigid than the steel design.

Steel Restraint

The steel-based restraint design hybridizes the previous designs, incorporating a magnetic restraint and an easy height adjustment mechanism. In this design, a 1" wide steel bar would be used to span the gap under the microscope objective. This would provide the most rigidity of all the designs since steel has a tensile strength of ~50,000 psi compared to the of ABS plastic with tensile strength of ~6,500 psi, a near order of magnitude larger. A hole would then be drilled in the steel bar to allow for the objective to image the mouse's brain. On each end an L-bracket would be attached and secured in a "ladder-like" device. This would allow the device to be easily slid in and out of different levels and the secured using bolts on each end. Indentations in the bottom of the steel bar would be used to fix the magnets in a method similar to that described in the wood restraint device. Overall, this device would provide the most rigidity of all the design alternatives, adjustable, easy to use, and cleanable. However, some difficulties with this design would involve its actual constructions, cutting and drilling through metal, and the costs associated with the special equipment required.

Design Matrix

The following design matrix was generated using the above design alternatives and criteria such as rigidity, adjustability, ease of use, ease of construction, ease of cleaning, microscope compatibility, and costs were assessed. It was determined that the steel-based design would perform the best with a total score of 91 out of 100, followed by the K'nex design with 83, and the wood design with 65.

	K'nex (ABS plastic)	Wood	Steel
Rigidity (35)	20	15	35
Adjustability (15)	13	13	15
Ease of Use (15)	15	10	13
Ease of Construction (10)	10	7	5
Ease of Cleaning (10)	10	5	10
Microscope Compatibility (10)	10	10	10
Cost (5)	5	5	3
Total (100)	83	65	91

Final Head Restraint Design

Upon determining that a steel design would best serve for our project, we began to look for a method by which to construct it. Since our client wanted a coarse form of adjustment for the head restraint due to the fact that the microscope's stage had fine adjustment, we were looking to create a "ladder" of sorts to place a bar that would span the distance across the stage. Our main restrictions were adjustability and size. The frame had to be easily adjustable, ideally by hand, and fit on the microscope stage.

We began by trying to figure out a method of making a "ladder." In searching at various stores, we came upon a plastic drain grate that looked to serve our purpose (Fig. 11).

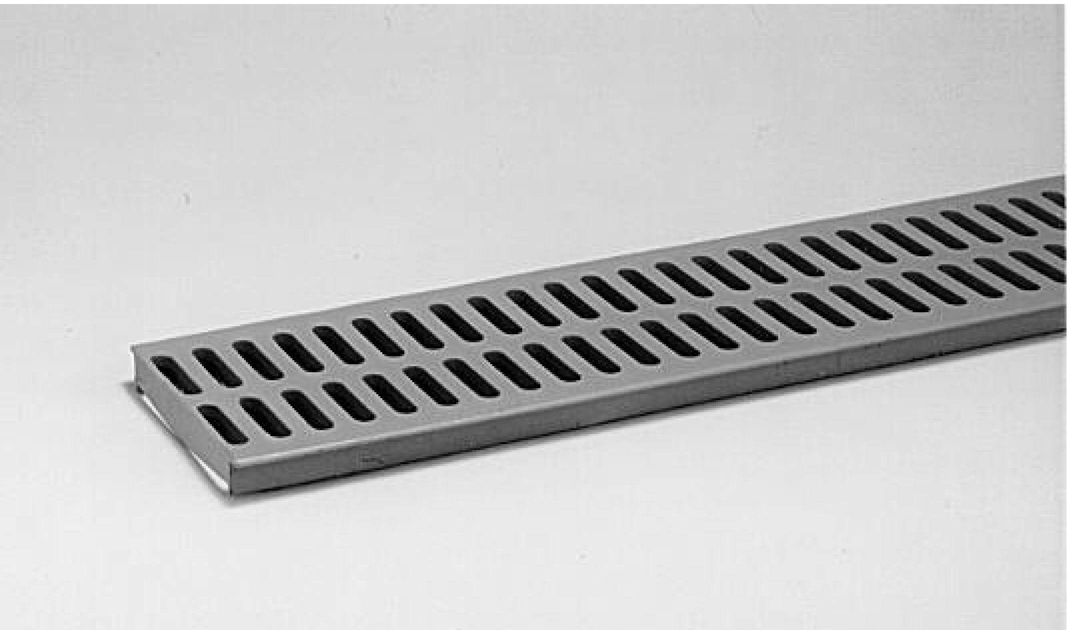


Figure 11. A sample channel grate, made of an unspecified hard plastic.
(http://www.connectorkings.com/jpeg/4-ChanGrate.jpg)

The grate was then cut lengthwise just to the side of the middle, to produce a strip of plastic with "rungs" on one side that opened outward (Fig. 12). These ladders were mounted onto a Plexiglas plate measuring 4" square, which was mounted onto the stage of the microscope.

We then purchased a 12-gauge steel bar, measuring 12" x 1", to use as the mounting base for the head restraint. Two 16-gauge steel L-brackets were also purchased to secure the bar to the ladder. The brackets were attached to the bar using two machine bolts, and further secured with cyanoacrylate glue. The completed assembly was then attached to the ladder using bolts and washers as shown in Figure 13. Though we originally used hex bolts, these were later replaced with eye-bolts and washers for easier changes, since eye-bolts are easier to turn by hand.

A staggered placement of the bolts is used to eliminate movement horizontally, either by twisting or going back and forth.

The steel bar was further modified to allow proper use of the microscope. A ½" diameter hole was drilled through the point over which the lens of the microscope would be, to allow for the laser to enter the cranial window of the mouse. In order to complete the head restraint, neodymium magnets were purchased and will be placed on the underside of the bar, as well as in dental cement seal of the cranial window.

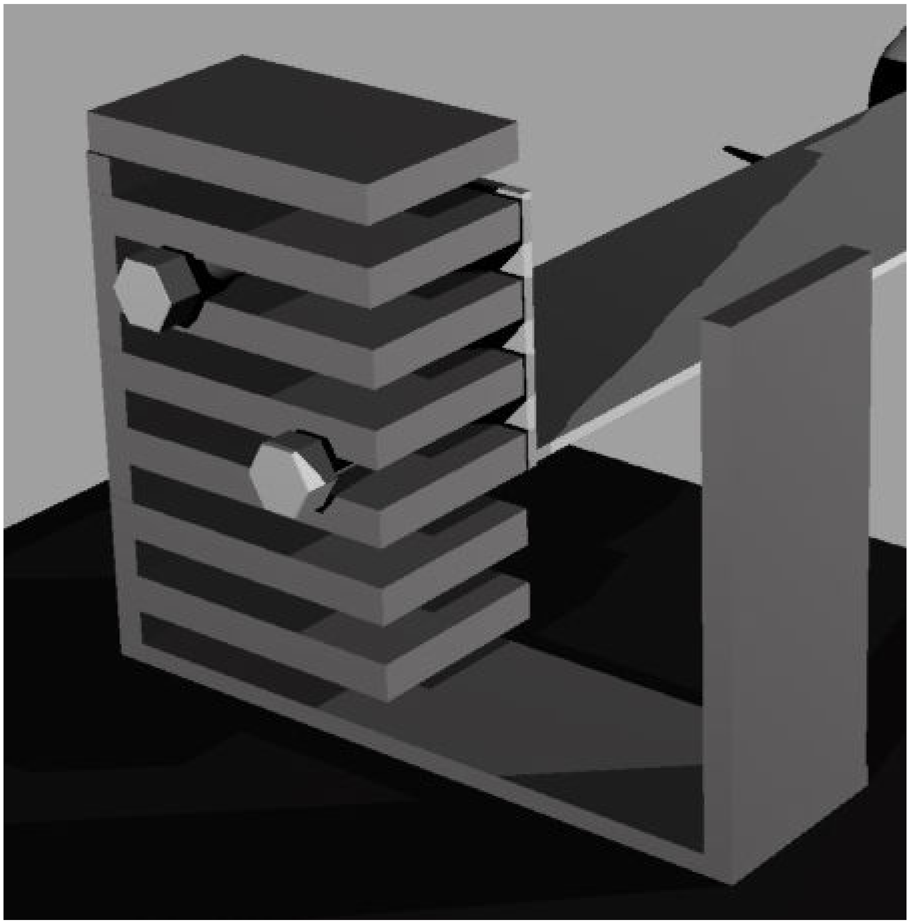


Figure 12. A close-up view of the completed "ladder" design. The hex bolts were replaced with eye-bolts for ease of use, and washers were placed on the nearest face to ensure an even spread of the force on the "ladder."

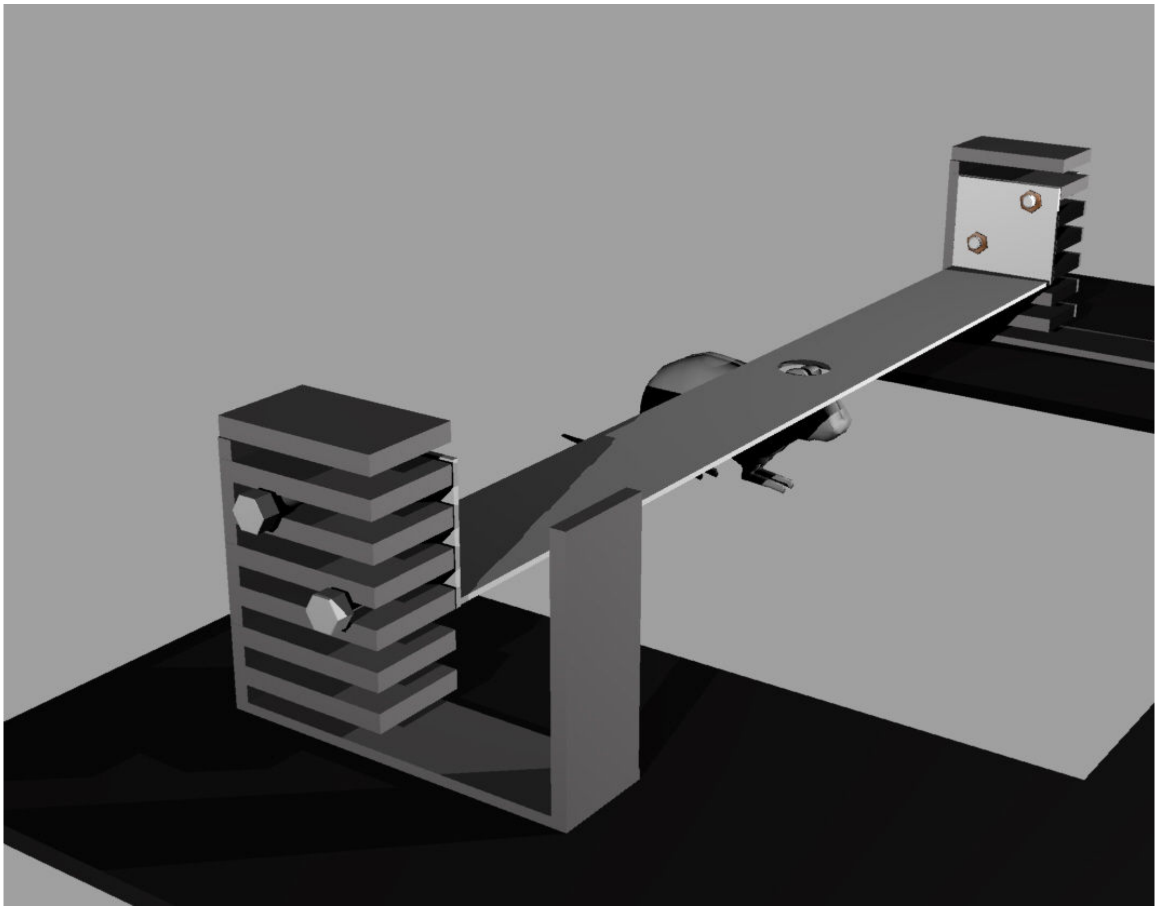


Figure 13. Shows the final construction of the head-restraint device, with the imaging hole drilled, and the entire construction mounted on the stage of the microscope. A mouse is included to demonstrate how the device would work. The mouse will be anchored to the bar using magnets. The hex bolts have been replaced with eye-bolts and washers, as mentioned previously.

Synergy of Ball and Head Restraint

After we had finished building all the components of the two designs we were able to attach the various parts to the actual microscope. This involved attaching the components to plexi glass. The two sheets of plexi glass that the treadmill parts were attached to needed L slots (1/4 inch) milled into them in order to allow quick adjustment of the placement of the treadmill device. This was not necessary for the head restraint since the client decided no horizontal adjustment was necessary due to the stage being adjustable already. The linear track actuator and the opposite side support were attached to the plexi glass with ¼ inch bolts. Holes were drilled through the plexi glass and counter sunk so that when the bolts were put into the plexi glass from the bottom into the actuator track and the support, it still sat flush against the microscope table. The linear actuator had holes on the side of it to mount L brackets which were then attached with ¼ inch screws to the plexi glass. The head restraint also utilized L brackets to affix itself to the plexi glass. The plexi glass itself was fixated to the microscope table with ¼ inch screws that fit into the pre drilled, one inch grid on the microscope table.

Testing on Final Prototype

The testing performed on our device was not exhaustive, but it demonstrated the effectiveness of the product. Using problems encountered with previous prototypes, there were only two major criteria in testing: ease of rotation of the ball, and ability for the mouse to be placed comfortably in position. Both of these were tested empirically, using a live mouse handled by our client, since this type of rough animal testing.

To test the ball's rotation, a mouse, held by its tail, was placed on the ball and agitated slightly. The mouse was able to easily rotate the ball using only its front two paws, and thus demonstrated that our ball could rotate. This test was repeated using a juvenile mouse, which was substantially weaker, with the same result. This confirmed the ability of the ball to rotate easily.

To test the ability for the mouse to be placed comfortably in position, we first had to install the head restraint and movement system in the microscope. The client then took a mouse and placed it on the ball, underneath the objective hole, and placed one prong of vinyl-tipped tongs through the hole, and held the mouse by the back of the neck. This simulated the restraining of the head. The mouse, while stressed, was able to run on the surface of the ball easily as well as stop and stand still. Since the mouse had not been trained, it was unable to rest, but this test demonstrated that the device functioned as desired by our client. Our client then repeated the test with a juvenile mouse. While there were slightly more difficulties in adjusting the ball to the best height for the tongs, the mouse itself was still able to run and stop similarly.

As such, our empirical testing methods demonstrated the effectiveness and utility of our device, making it suitable to our client's needs.

Future Work

The project is being used right now with the client and the only future work would be to make any adjustments that arise after the client has used the device more. The tests that were done on the final design were not very exhaustive tests that looked promising. Due to the mice not yet being trained on the device or having the magnets adhered to their skull no true testing could be done.

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

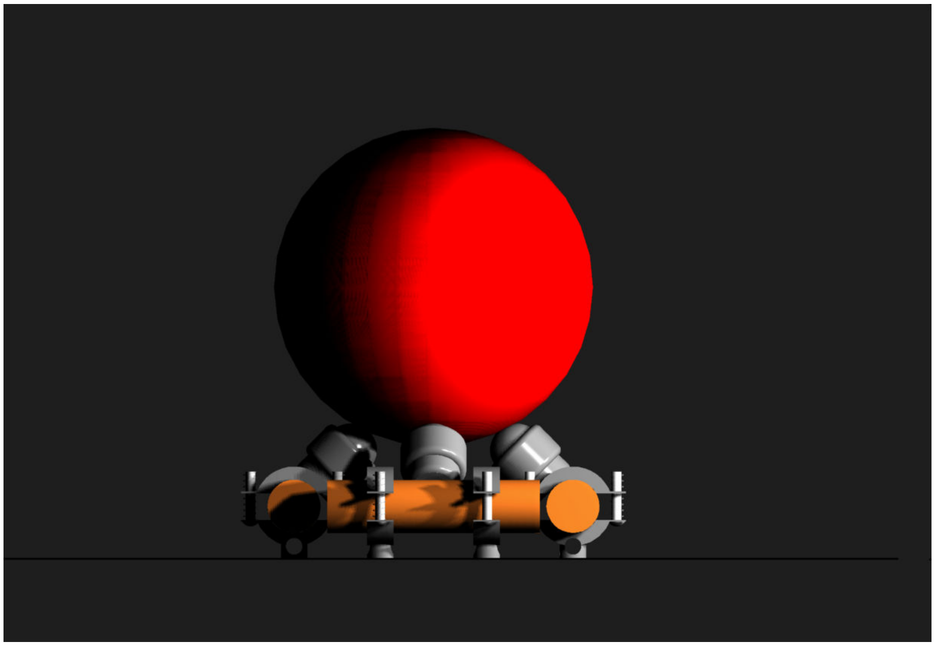
Expenses

Item	Store	Card Number	Cost of item	Total w/ Tax
	Home Depot	xxxxxxx7347		$18.48
Sheet Metal			$5.84	
Grnd Clamp			$11.68	
	Home Depot	xxxxxxx7347		$12.74
150mm Orment			$3.99	
Undercoat			$4.97	
Spray paint			$3.12	
	Menards	xxxxxxx1750		$3.15
150mm Ornment			$2.99	
	Wisconsin Craft Market	xxxxxxx1750		$1.89
	Wisconsin Craft Market	xxxxxxx7347		$20.67
	Ace Hardware	xxxxxxx7347		$4.22
Hardware			$2.25	
Hardware			$1.25	
Hardware			$0.50	
	Home Depot	xxxxxxx7347		$9.19
3/8 HBlt			$0.19	
3/8 Hexnut			$0.18	
Bev+Necdep			$2.76	
3/8 RNRD			$5.58	
	RC Performance	xxxxxxx7347		$41.12
Gears x9			$38.98	
	Wisconsin Craft Market	xxxxxxx1750		$13.98
	Home Depot	xxxxxxx7347		$20.49
Comp. Spring			$3.98	
Metal Blade			$4.97	
Saw Blade			$5.47	
.093 11x14AC			$5.00	
	Home Depot	xxxxxxx6580		$64.10
Flats			$5.74	
8ft Ext kit			$47.56	

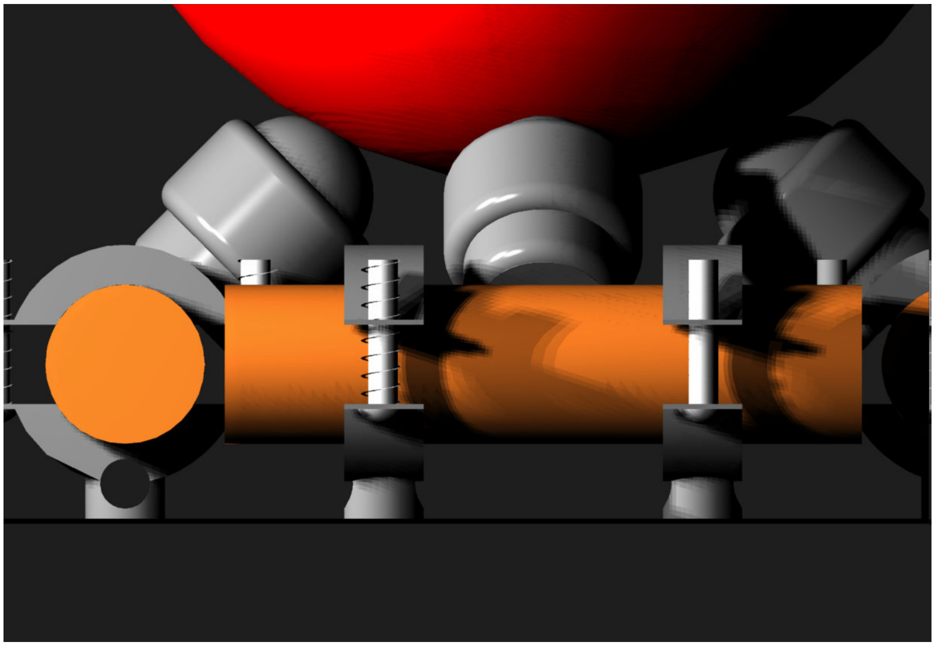
Thrded Rod			$2.98	
Comp Spring			$4.48	
	VXB Ballbearing	xxxxxxx2673		$70.20
1/2" Mounted			$36.25	
10x Shielded			$33.95	
	VXB Ballbearing	xxxxxxx7347		$90.99
Bolt Type x10			$29.95	
10 Loose Ball			$39.90	
	Misc. Items			$50.00
Total				**$421.22**

Appendix C

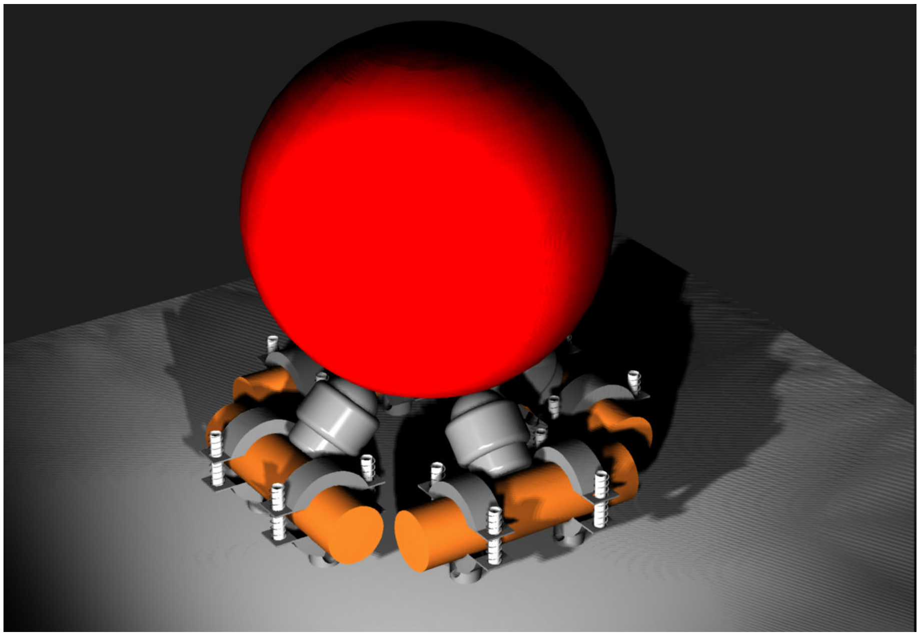
Final Prototype Design 1



Global view of final design



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

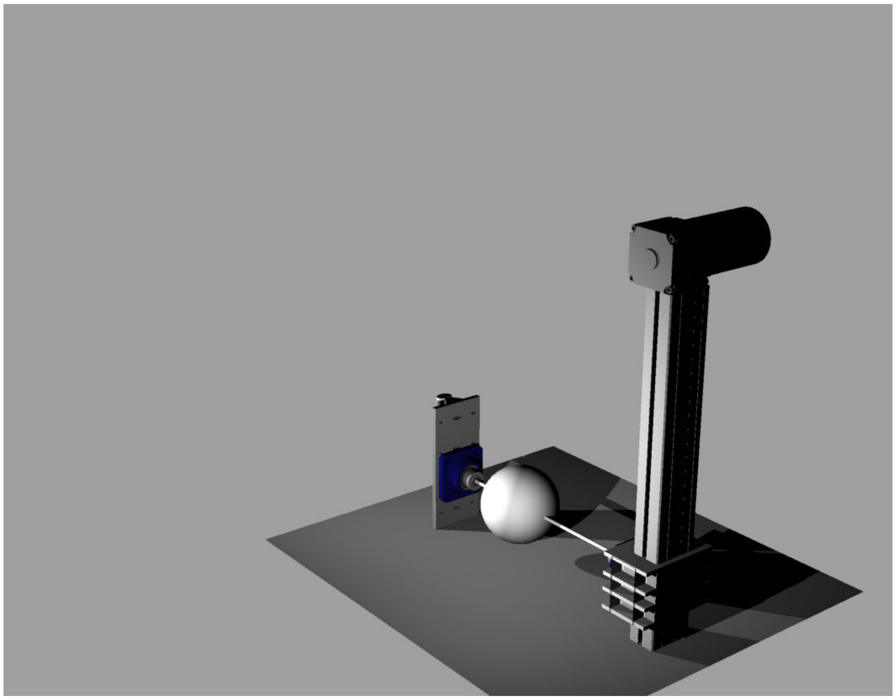


Birds eye view of prototype design. Note how the electrical clamps are put in upside down.

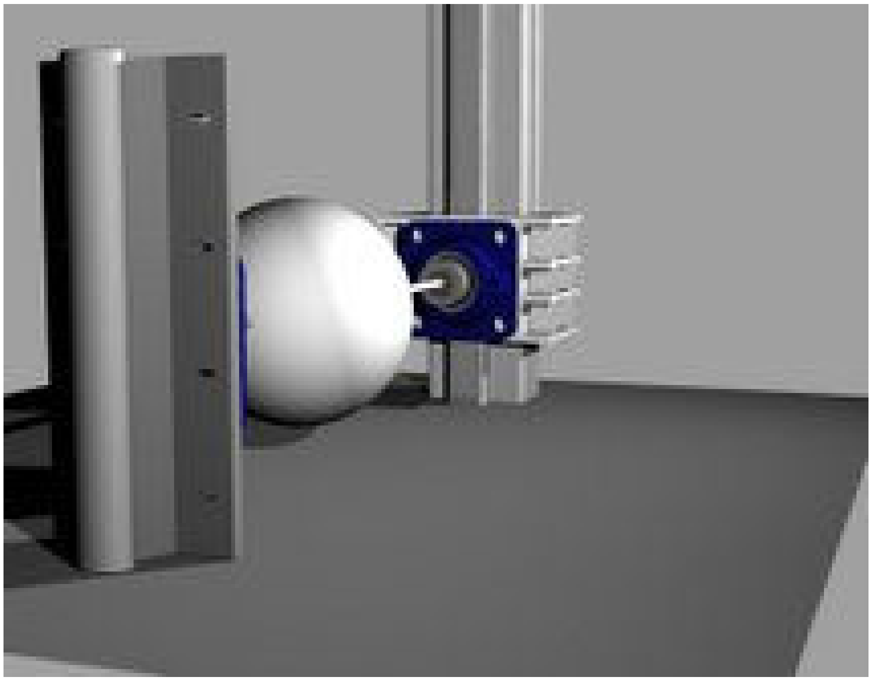
Appendix D

Second Final Prototype Phase 1

(Actual Design)



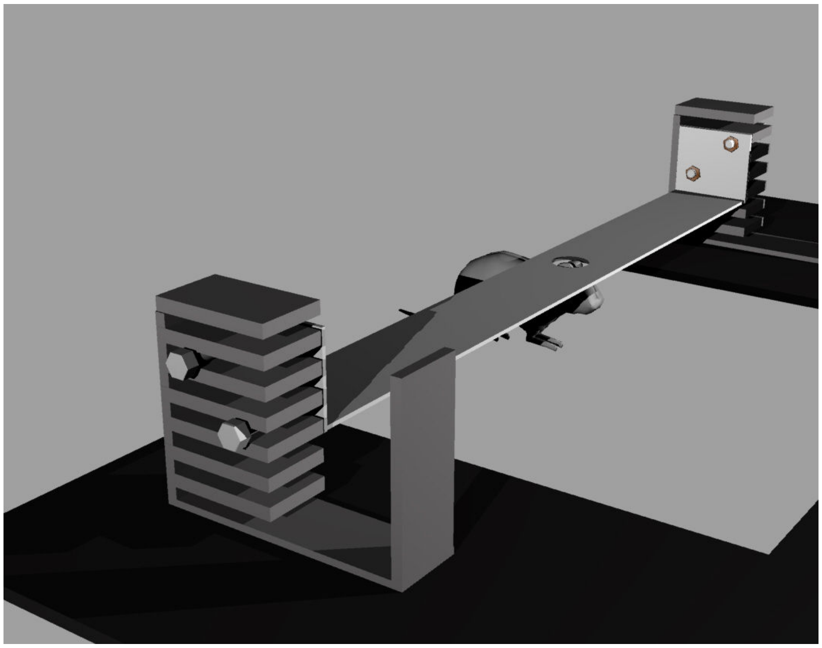
View of final design used by the client ("treadmill" design)



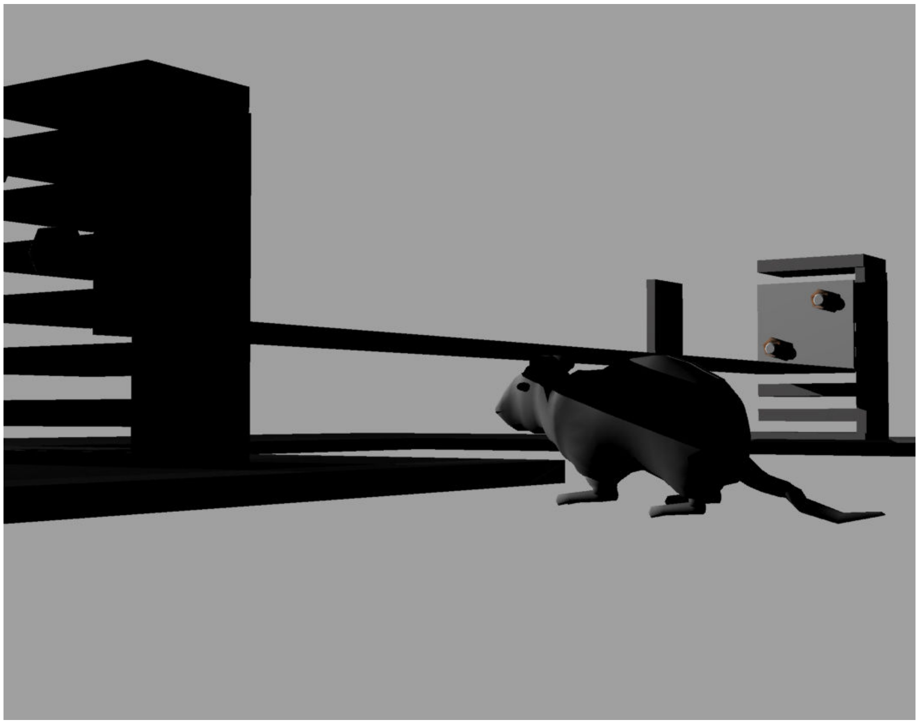
Close up of Bearing used to house Axle

Appendix E

Final Head Restraint Design, Phase 2



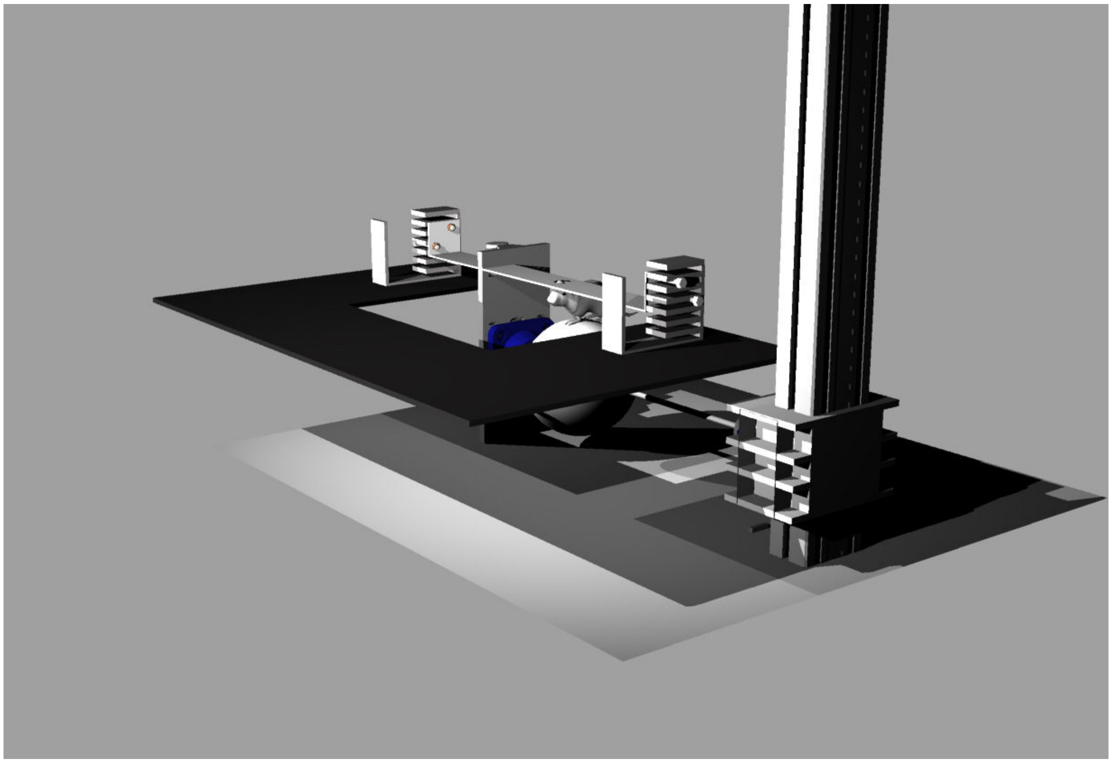
Head restraint from a side view. Seen is the adjustability aspect.



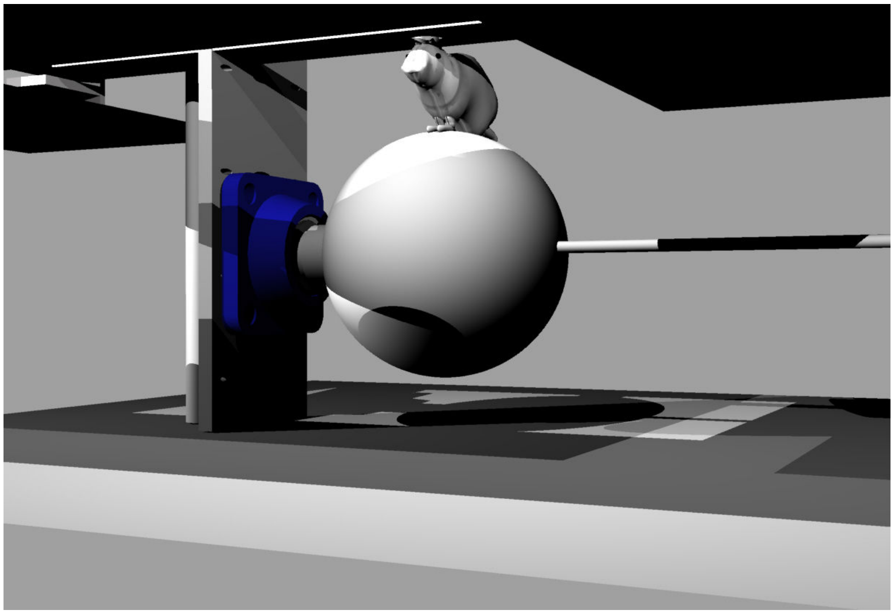
Close up view of head restraint with mouse.

Appendix F

Final Designs Combined



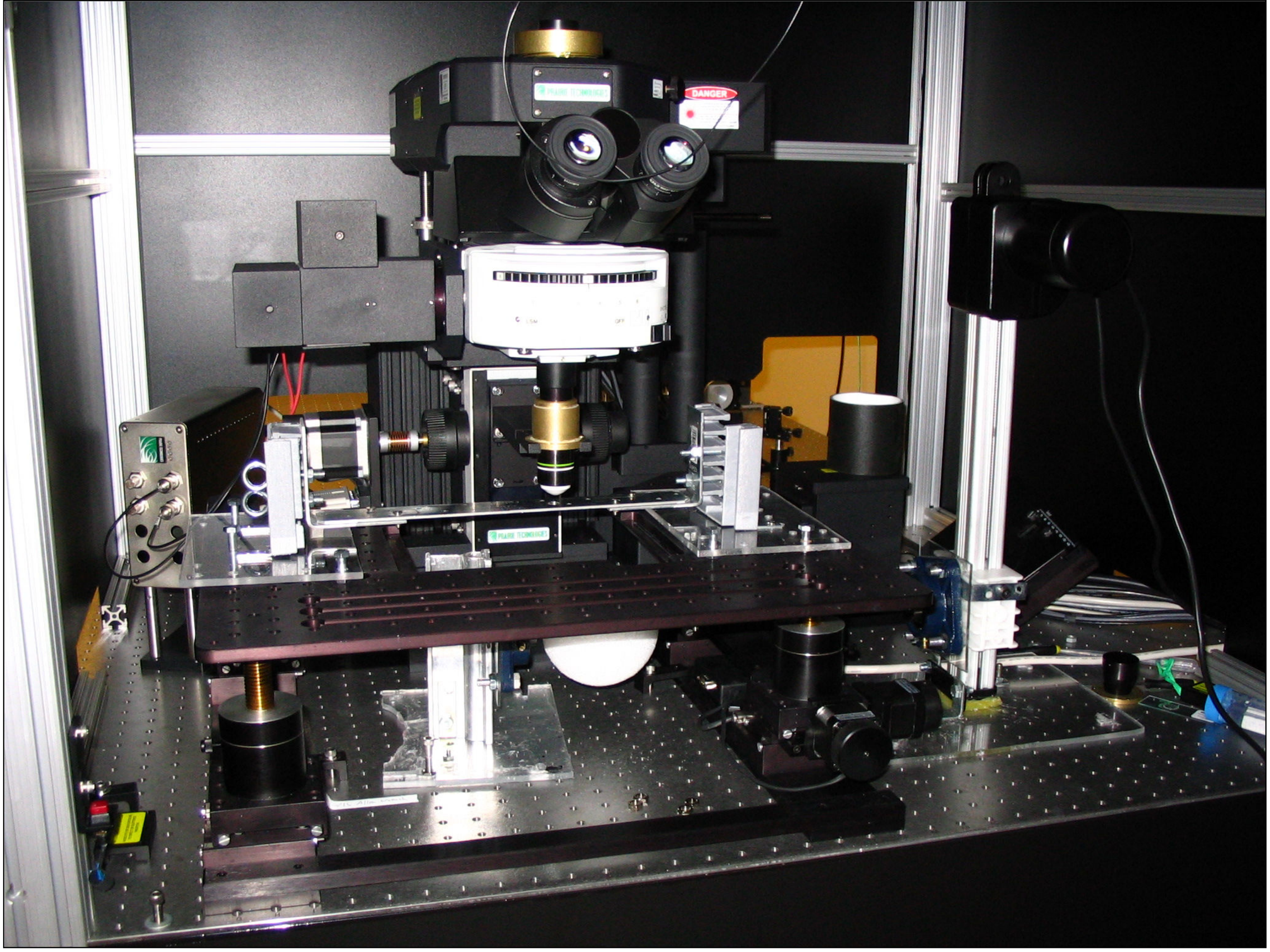
Final Design global view



Close up view of final design with mouse

Appendix G

Real Pictures of Complete Final Design


Final Design used by the client

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Turner, T. H., Drummond, S. P. A., Salamat, J. S., & Brown, G. G. (2007). “Effects of 42 hr sleep deprivation on component processes of verbal working memory.” *Neuropsychology, 21,* 787-795

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Dombeck DA, Khabbaz AN, Collman F, Adelman TL, Tank DW (2007) “Imaging large-scale neural activity with cellular resolution in awake, mobile mice.” *Neuron, 56,* 43-57.