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Rat Elbow Project

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Introduction

Current methods for treating post-traumatic joint stiffness (PTJS), a common clinical problem following elbow surgeries which causes functional impairment of the limb, have proven insufficient.[1] The main factors that expose the elbow joint to this complication are a high degree of congruence, the complexity of the joint surfaces and the high tissue sensitivity to trauma, especially the joint capsule.[1] As a result, the Lake laboratory is working with elbow tissue to determine how the injury presents itself. With a deeper understanding of the injury, the lab hopes to develop more effective prevention and treatment methods for PTJS.

Before the fall of 2016, the lab successfully developed an animal model for the injury which can lead to PTJS. This model utilizes surgically injured elbows of Long-Evans rats. Currently, the lab is using this model to further understand the injury. In order to explore the presentation of the injury, the lab would like to use gait analysis. Gait analysis, or the examination of the steps and stepping patterns of the rats, provides an effective method to test the consequences of physical and drug therapy in a noninvasive manner. This is because walking is a natural behavior and therefore can be used to compare an injured rat to control. Gait analysis quantifies the consequences of injuries and provides useful data which can be used to gain a deeper understanding of the injury. Before the fall of 2016, DigiGait, an automated gait analysis system, was used with minimal success. Thus, the laboratory decided to pursue other methods.

AGATHA

The lab decided to implement a system called AGATHA. This system has been used previously by Kyle D. Allen's lab at the University of Florida. AGATHA, Automated Gait Analysis Through Hues and Areas, is a fully-automated open-source method for the characterization of rodent spatiotemporal gait patterns.[2] It utilizes a camera system to collect information about the gait of the rat and then, using MatLab, translates that information into useful data. Unlike other gait analysis systems, significant work has gone into validating AGATHA against manual digitization to ensure its accuracy.[2] Additionally, while DigiGait was not effective, the lab believes that AGATHA's open-source nature will be more suitable for this project.

AGATHA requires a complex camera and arena set-up in order to be used effectively. My semester began by collecting the necessary information from the lab in Florida to correctly build this system, shown in Figure 1, ordering the parts, and then constructing it.
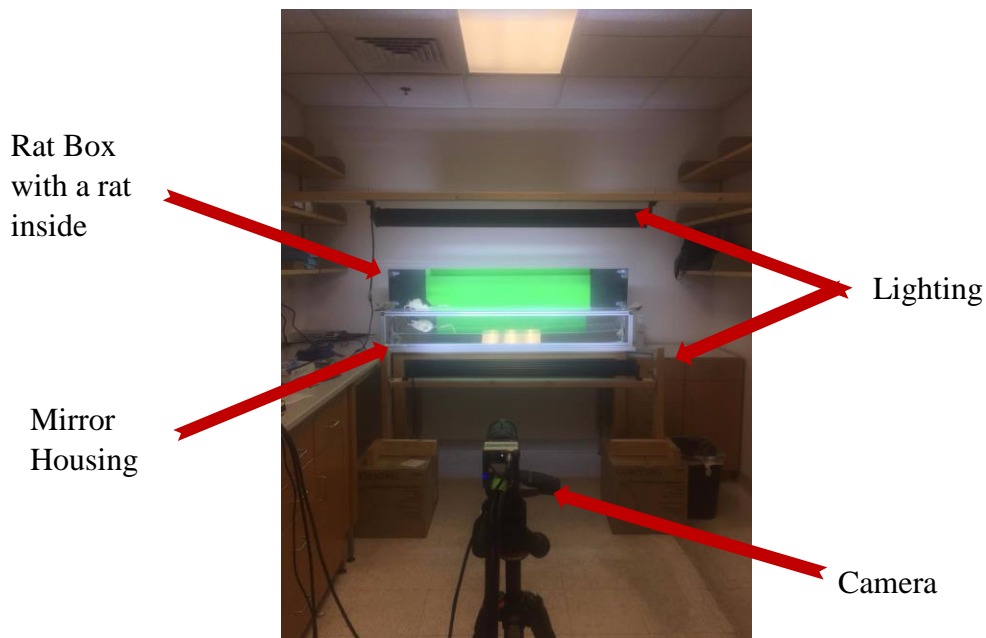

Figure 1: The AGATHA system in use with
a Long-Evans rat on the left-hand side.

The AGATHA system uses a single camera to collect both the sagittal view, the view from the underside, and a front on view of a walking rat. In order to do so AGATHA isolates the sagittal view of the animal and then looks for the silhouette of the animal in the video by subtracting an image of the structure without the animal in it.[2] After collecting this image, it locates the rat's feet to analyze the animal's gait. This is all done while the rat is freely walking in the structure. The ability for rats to walk freely makes them more comfortable during testing and is a major advantage over other gait analysis systems.

The structure itself is composed of two parts; a housing for the mirror and the rat box on the top which holds the rat during testing. The component that houses the mirror, displayed in Figure 2, was created using 80/20 bars, 80/20 corner brackets, 80/20 button head socket cap screws, a .25" thick acrylic sheet on the top to support the weight of the rat, and a mirror that runs the length of the structure. The mirror is the key component of the structure. Angled at 45 degrees, it allows the lab to collect the necessary front and sagittal views using a single camera. The 80/20

was used to ensure the structure was rigid enough to easily support the weight of the rat and not deform during testing.
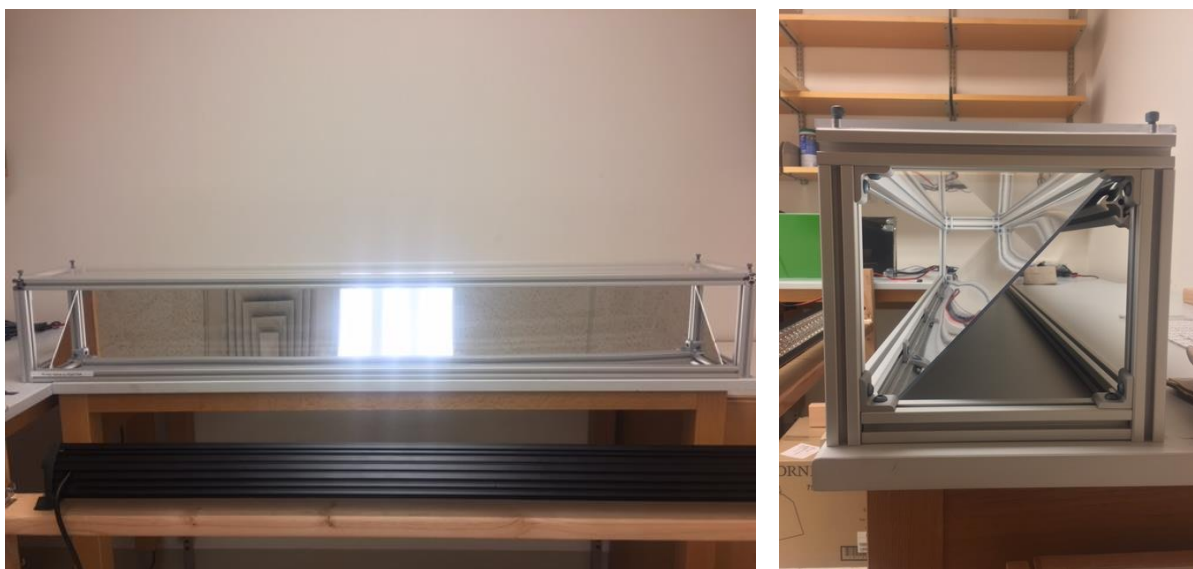

Figure 2: Front and Side View of the AGATHA's Mirror Housing Component

The rat box, shown in Figures 3 and 4 on the next page, rests on the top of the structure and was created using .125" thick acrylic sheets, corner brackets with screws and nuts, piano hinges, as well as green and black vinyl. The box is designed to be clear but rigid enough to hold the rat. Acrylic, a clear material, was used so that the camera can see the rat while it is walking. The green vinyl creates a contrast in the background of the image that the camera collects so that it can distinguish the rat from the structure. A lid with green vinyl was placed on the top of the box to provide contrast for the sagittal view. The black vinyl on either end of the enclosure provides the rat with a "safe zone". The rat is not being recorded in these areas. While the acrylic will serve the purposes that it needs to, if I were to replicate this project I would have used different materials in the construction of the rat box. Simply put the box is not as rigid as I would have liked it to be. Although the front of the enclosure would need to remain clear, I wish I would have created the other sides of the box using wood, a far sturdier and less expensive material.

Figure 3: Rat Box Front View   Figure 4: Rat Box Side View

Light bars for a jeep, shown in Figure 1, were used to provide consistent lighting. Good lighting is an important part of minimizing error and false readings while using the AGATHA code. These lights were placed both above and below the system and were attached to wood supports to ensure their stability.

Now that I have successfully constructed the AGATHA arena the lab may begin using it for testing next semester. Gait analysis will be used by the lab to analyze the effectiveness of both drug and physical therapy. Following my work on the AGATHA structure I began work on creating a system that could be used for physical therapy.

Physical Therapy System

Walking, a simple method of physical therapy, will be used to treat the injured rats. In order to ensure consistency across testing, the laboratory decided to use a treadmill system to regulate this activity. I spent many hours finding the correct treadmill. We wanted a treadmill that we could modify to have a suitable speed for the rats to walk at as well as small speed increments to allow for variance during testing. The ability to change the incline of the treadmill was also important. Although it may not be used in initial testing, it is possible that varying incline will be used in future research. We had a target speed range of about 10-40 cm/s, a range

that was determined by Alex Reiter after working with the rats on the DigiGait system. Additionally, we wanted to be able to set the treadmill at enough speeds in this range to allow for adequate data collection.

With this information I purchased the horizon T101-4 treadmill, which is shown in Figure 5. This treadmill has a maximum speed of .5 mph (22.352 cm/s) - 10 mph (447.04 cm/s) with increments of .1mph (4.4704 cm/s). This treadmill can also be inclined from 1-10% incline using 1% increments.

Figure 5: The Horizon T101-4 Treadmill

Obviously, the speeds of this treadmill do not satisfy the target range so modifications will need to be made. In order to successfully slow down the treadmill I designed a 2 piece part that will be attached around the wheel that is attached to the motor by a belt shown in Figure 6 on the following page.
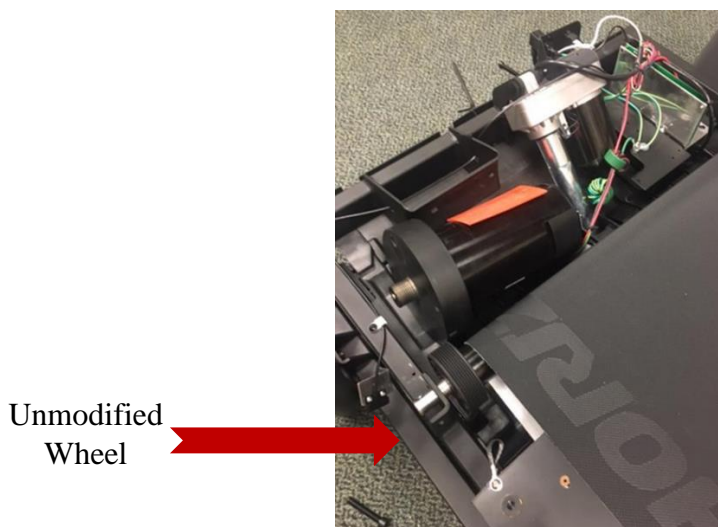
Unmodified
Wheel

Figure 6: View of the motor and unmodified wheel.

To slow down the treadmill to the correct speed it was determined that the radius of this wheel must be doubled. By doubling the radius we will cut the angular velocity of the treadmill in half which will induce the desired speed range. The existing radius of the wheel is 3.49 cm. Using V=wr to convert from linear velocity to angular velocity and doubling the radius to 6.98 cm will allow the treadmill to operate at a range of approximately 11.176 cm/s – 223.52 cm/s in increments of 2.2352 cm/s. We project that this range will allow for data to be collected at 13 different speeds. After the modification are completed a tachometer will be used to ensure the target speed range was reached.

The part to modify the wheel was created on Inventor, a computer aided design program, and will be 3D printed. Shown in Figure 7 I designed the part as two simple semicircles. This part will wrap around the existing wheel. First, I made designed the grooves on the inner semicircle to fit into the grooves on the existing wheel, just as the belt currently does. I then designed the grooves on the outside of the wheel to by symmetric with the grooves of the existing wheel to allow the belt to grip to the new part just as it did the old one. The two halves of the part will be attached using screws and nuts. Extrusions on each side of the part will allow these screws and nuts to successfully hold the two halves together without coming into contact with the belt as it spins. This is important because it will allow the treadmill to run as smoothly as it did before modifications.
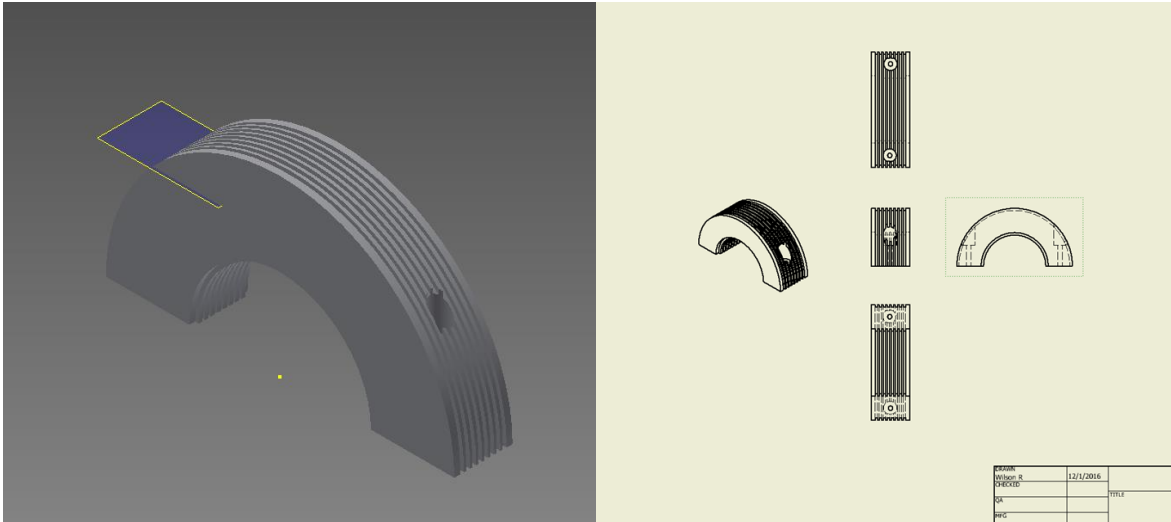
Figure 7 Designs and sketches of the modified wheel.

To complete the physical therapy system, an enclosure that will house the rats while they are walking will be built. I modified designs of a structure that Dr. Lake used in his previous lab which will be used to create this enclosure. As shown in Figure 8 and 9, photos taken by Snehal Shetye, there will be two separate but identical enclosures. Each of these enclosures will hold four rats at a time. This will allow for eight rats to undergo testing at once.
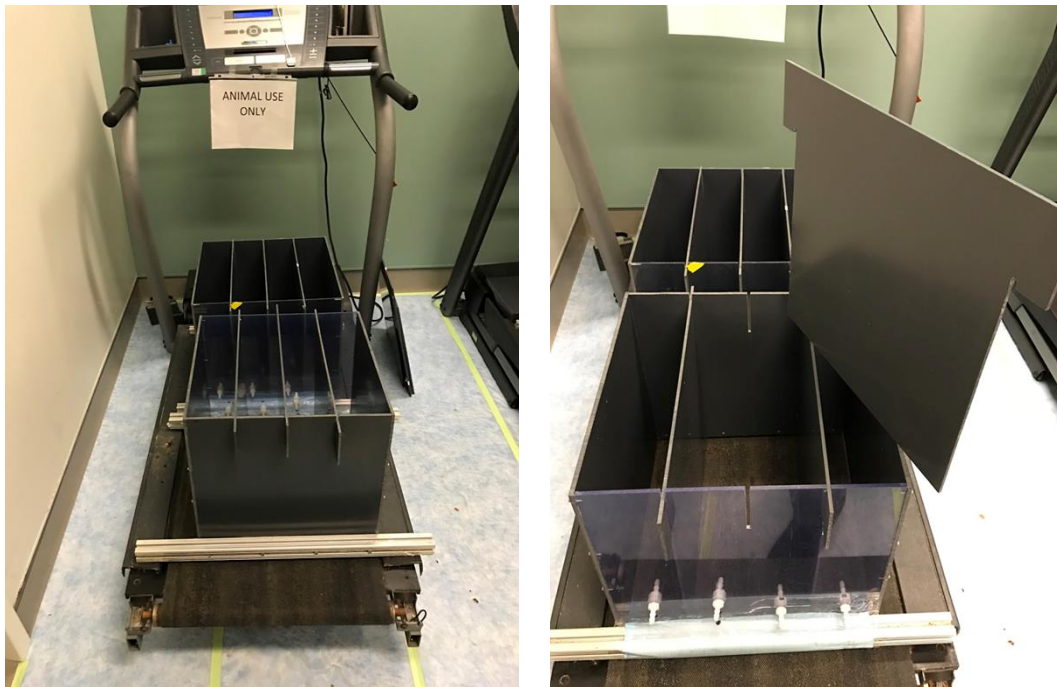
Figures 8 and 9: Physical therapy system example photos taken by Snehal Shetye.

The enclosure will be made out of black acrylic sheets and 80/20. Black acrylic is used so that the rats cannot see eachother while walking. The boxes themselves are designed to be easily cleaned after testing. The innner walls of the boxes are can be easily lifted from the slits they rest in by the experimenter to be cleaned. Additionally, the enclosures are designed to rest slightly above the treadmill belt. This gap between the enclosure and the belt serves two purposes. First, it ensures that the belt is able to run smoothly. Second, it will allow anything that will need to be cleaned to be carried off of the back of the treadmill and not get stuck in the enclosures. In order to create this gap the boxes will be attached to the 80/20 bars in the front and the back. These 80/20 bars will then be attached to the treadmill outside of and above where the belt spins. Each lane is designed to be wide enough for the rats to be able to fit in comfortably. However, the lanes are also small enough to encourage them to walk forward. Multiple methods will be used in order to encourage the rats to walk if necessary. As seen in the figures above, compressed air connections can easily be attached to the box in order to motivate the rats. Other methods, such as the use of a prod or treats may also be used.

Results & Discussion

My initial project, the AGATHA structure, was completed. Currently, the lab is working with the creator of the MatLab code, Kyle Allen, to ensure that the code will work correctly for this project. Modifications are expected to be made. For example, the existing code has only been used for all white or all black rats. Long-Evans rats are both white and black which may cause difficulties in the image collection process. However, after the code is tweaked, the system will be ready for use!

I completed initial designs for the physical therapy system as well. Unfortunately, I will not be able to see this project completed. The part for modifying the wheel will first be 3-D printed on a low fill to test. If testing goes well the part will be reprinted at a higher fill for use. The parts for the creation of the enclosures have been ordered and they will simply need to be assembled when the parts arrive.

Conclusion

In conclusion, significant progress has been made on the Rat Elbow Project this semester. The AGATHA system will provide a reliable and accurate way for the lab to evaluate physical

and drug therapy on rats' injured limbs in the future. This system will hopefully be a significant improvement over previously used methods and will likely be used through the project's completion. The data collected using the AGATHA system will hopefully lead to further revelations and research into PTJS by Dr. Lake's lab as they search for more effective treatment methods.

I was hopeful that I would be able to complete the physical therapy system as well. However, locating and purchasing the correct treadmill for the project took significantly more time than planned as it had fairly specific requirements. However, I believe that my contributions should allow for this project to be completed soon. With the design portion of the project completed, other members of the Musculoskeletal Soft Tissue Labratory including Alex Reiter, will complete the project by constructing and testing the system.

After these projects are finished the lab will continue exploring the presentation of PTJS following elbow injuries. In the short term this will include significant testing using the AGATHA and physical therapy systems. After ample data has been collected the lab hopes to be able to develop a prevention/treatment strategy for elbow PTJS.[3]

References

[1] G. M. Filh and M. V. Galvão, "POST-TRAUMATIC STIFFNESS OF THE ELBOW," vol. 45, no. 4, Nov. 2015. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4799099/. Accessed: Dec. 10, 2016.

[2] H. E. Kloefkorn et al., "Automated gait analysis through hues and areas (AGATHA): A method to characterize the Spatiotemporal pattern of rat gait," Annals of Biomedical Engineering, Aug. 2016.

[3] S. Lake, "Elbow Stiffness," in Musculoskeletal Soft Tissue Labratory. [Online]. Available: http://lake.seas.wustl.edu/research/elbowstiffness/. Accessed: Dec. 10, 2016.