Uniaxial force plate for the locomotive mechanics of lab rats and their neural feedback response

A Design Document
Submitted to
the College of Engineering Faculty

In Partial Fulfillment
of the Requirements for the Degree
Bachelor of Science

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by
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Diploma Date: May 2017

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Abstract

SD2: Team 10	Doctor DREADD
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Project Title	Uniaxial Force Plate for the Locomotive Mechanics of Lab Rats and their Nueural Feedback Response
Department	Bioengineering
Abstract	This senior design project describes the research, ideation, design, construction, and implementation of a mechanical data acquisition system that will have the ability to measure the mechanical feedback response of mice during locomotion. By doing this we will be able to collect information about the neuromuscular response of the mice during locomotion and how having feeling affects this feedback response. By creating a one dimensional force plate that can measure the dynamic response of mice on a treadmill where we can create a waveform that describes the application of force by the mouse paw onto the force plate. In order to create a experimental group without feeling in their paws we will use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) which will have the effect of turning off that feedback response in the mice.

Executive Summary

Spinal Cord Injury (SCI) induces locomotor deficits responsible for significantly impairing functional abilities. Recent studies in the field of neuroscience have focused on neural stimulation techniques for post-SCI rehabilitation. Superior instrumentation is necessary for testing the affects of these techniques, as traditional motion capture systems may not be able to accurately detect motor response.

Our project requires that we design and construct a uniaxial force plate that can be utilized to test rat limb forces to aid in spinal chord research. The purpose of this project is to provide a means of validation for the effects of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). The hM4Di DREADDs are inhibitory constructs that have been studied in rodent models for manipulation of the feedback response. Such research facilitates analysis of neural circuits and how they regulate behavior, which ultimately fosters a better understanding of the necessities of an effective SCI treatment.

The decision to focus on a uniaxial force plate rather than a 3-dimensional design was made based on the difficulties experience by last year's team. While a 3-dimensional design is desirable, optimizing results in one axis will allow future design teams to include the additional dimensions.

There are several design requirements that our force plate must satisfy. The plate must be an appropriate size to accommodate lab rats, approximately 102 by 204 mm. Simultaneously, the plate must be small enough to retain the sensitivity required to accurately record the forces put down by the rats' limbs. Our design utilizes deflections of cantilever beams to record rat limb forces. To collect data, we will use Omega strain gauges, thus requiring that the forces translate to at least 10 microstrains. In addition to optimizing the force plate's functionality, we must provide a method for enticing the rats to run accurately across the force plate. To achieve this, we have designed a setup incorporating a hallway ensuring the rats must run across the surface of the force plate to get from one end of the setup to the other.

Such force plates have been designed and implemented before, however the cost of a force plate sufficient for recording rodent force data ranges from $2000 to $10000. In our design, we aim to not only create an accurate uniaxial force plate, but also lower the cost of creating such a plate exponentially by remaining well within our 1000 dollar budget. This will be achieved by choosing to 3D print the legs of the force plate and using strain gauges that are relatively inexpensive for the ease of installation and accuracy they provide.

Contents

1 Problem

1.1 Initial Problem Description

Spinal cord injuries debilitate people by limiting the functionality of the central nervous system due to damage to spinal cord. Currently over 17000 new spinal cord injuries (SCI) are reported each year and they lead to restriction of motor functions such as walking or organ function in those affected (Center, 2016). Some of the most bleeding edge work in the biomedical field is to get the cells that have been damaged to function again. Currently used methods have adverse side-effects because they involve electrical stimulation of fine motor control neurons which cause involuntary activation of motor neurons of unintended areas.

Chemogenetic drugs which exploit drug delivery methods that use viruses to change the genetic makeup of damaged cells are one alternative solution but they need verified testing procedures to determine how effective they are. The process of using small mammals to observe the apparent effect of a drug is one such necessary practice in the field of gait analysis. This kind of testing allows for the relatively quick testing of the effect of new drugs or treatments by using a control group of animals with a injury to the muscular-skeletal system or nervous system and comparing their movement to a group of animals with the same injuries but with the addition of the drug or treatment.

However, because of the instincts of small mammals such as rats, there will inevitably be a effort on their part to mask their injuries. Imagine a rat with an injury, an injury to the nervous system that affects its gait to the point where it is noticeably limping or dragging its feet. This rat will be a target for predators so it is only it's natural instinct to mask this injury. As a result, using the qualitative evidence from the kinematics of the animal will not always provide substantial evidence that the drug or treatment is actually effective. To account for this behavior scientists who conduct gait analysis use kinetic tests using equipment such as force plates to get quantitative force data that cannot be hidden by the rodent. These pieces of equipment are able to pick up the discrepancies in the forces applied by the four limbs of the rat in the $X, Y, \& Z$ direction using sensors that have a definable relationship between their output signal and the force applied.

1.2 Overall Analysis and Objectives

To better treat patients with SCIs, rodent testing must be conducted first that confirms that the drug or treatment helps restore function and feeling in muscles. This project's goal is to test a certain set of Chemogenetic Drugs that work by using designer receptors exclusively activated by designer drugs (DREADDs) using kinetic testing equipment in the form of a force plate that measures force in the vertical direction.

Chemogentic drugs are drugs that are delivered using mechanisms such as viruses to introduce genetic material to cells in the body to make them reactive to the presence of designed chemical compounds.The chemogenetic tool we are using is called Clozapine-N-oxide activates Gq-mediated signaling or hM3dg. This alteration in the genetic material of the cell is activated using Clozapene-N-Oxide or CNO to cause a rapid firing of the nerve cell that was previously not functioning. It is believed that with repeated use that this practice could cause the regeneration of nervous system function after a SCI ().

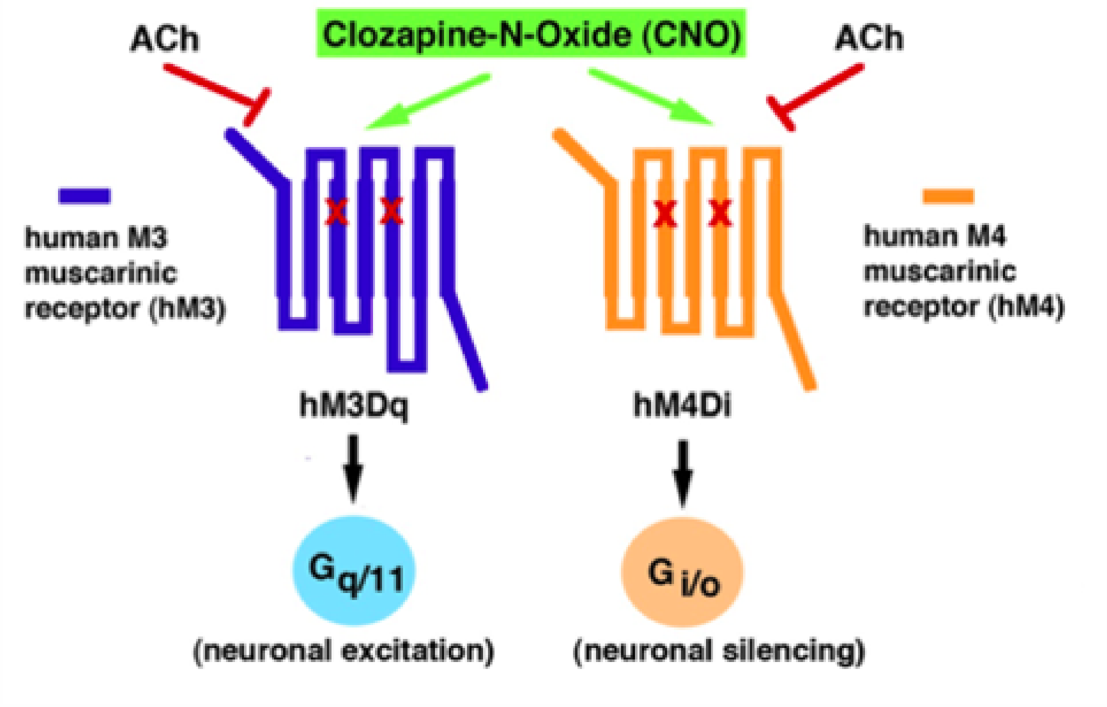

Figure 1: Excititory and Inhibitory DREADDs Diagram

Because rats and other small mammals are prone to hiding their injuries due to instinct a method of verifying the effect of the drug that depends on measuring the forces applied by their limbs. One method of doing this is by using a force plate that A force plate is a piece of equipment used in gait analysis that is able to resolve the forces applied on a platform. While there are many different kinds of force-plates a well designed force-plate should abide by at least these six criteria:

1. Have sufficient sensitivity and resolution for the subject of interest
2. Have a linear response to the force that is exerted
3. Have a response independent of where on the plate surface the force is exerted
4. Have a high natural frequency of oscillation
5. Have a sufficient coefficient of safety to protect the equipment and specimen.
6. Have a reasonable cost and operation procedure

(Heglund, n.d.)

The goal of this team is to create a force-plate that satisfies the criteria above for an experiment measuring the force of rats running across a track. This force plate will measure the force in the vertical direction in order to verify the effect of the DREADDs even if the animal makes the effort to conceal their injury.

1.3 Historical & Economic Perspective

SCI has been rated as the second most costly illness to treat in the United States (Winslow et al., 2002). According to the Centers for Disease Control, U.S expends approximately 9.7 billion dollars annually for SCI treatments. Individual SCI patient costs reach up to 1 million, including hospital fees, home adjustment costs, and medicinal supplies and personal assistance expenses (Mckinley et al., 1999).Additionally, the Spinal Cord Injury Research Program (SCIRP), established by Congress, is funded 30 million dollars to support research for SCI therapies (http://cdmrp.army.mil/scirp/default). Upon economic evaluation, it is clear that SCI is expensive in maintenance, treatment and research and that cost-preventative approaches are necessary for lowering total costs.

Research costs can be ameliorated through innovative development of inexpensive testing hardware and instrumentation. Commercial force plates can range higher than $20,000. With such costly equipment, less developed countries will not be able to conduct research and support patients with SCI to their fullest extent. To ensure global advancement in the field of SCI, instruments such as force plates need to be manufactured at minimal expense.

1.4 Candidate Solutions

1.4.1 Support Material and Structure

Material	Yield Strength (MPa)	Density($\frac{g}{cm^3}$)	Modulus of Elasticity (M
Brass	220	8.960	$115.0X10^3$
ABS Plastic	80	1.060	$3.5X10^3$
Rigid Opaque Plastic	60	0.970	$3.0X10^3$

1.4.2 Sensors

Sensor	Linearity	Stability	Cost
Strain Gauge	Yes	Temperature Sensitive	$ 5.00
Piezoelectric	Not over 3N		$36.00
Hall Effect Sensor	Yes	Yes	
Optical Sensor	Yes	Yes with correct alignment	$0.00

1.5 Proposed Solutions

Based on our findings, we made the decision to construct the legs of the force plate using ABS plastic. While brass has been used in the past for similar designs, it is expensive and difficult to machine. Our goal is to keep costs low and create a simple enough design that other labs can easily replicate given the specifications. By choosing ABS plastic, we are using a material that can be printed using a 3D printer and that is strong enough to meet our required specifications.

1.6 Major Design & Implementation Challenges

1.7 Implications of Project Success

2 Design Requirement

2.1 Target Specification

2.2 Final Specification

3 Approach

3.1 Introduction

3.2 Support Design

3.2.1 Structure Geometry

The design of the structure is dependent on theory outlined in the paper by Norman C. Heglund which describes the creation of a force plate that operates using strain gauges on spring blades to measure the force applied. This method follows all of the criteria for a force plate that are outlined in the overall analysis and objectives portion of the design document. This design is based off of 3 key elements including how the spring blades are modeled as two cantilevered beams that are joined in the middle and the measurements are then calculated for one cantilevered beam. The beam that connects the two spring blades also need to be taken into consideration in order to be able to be used for the specific study that will be conducted with the laboratory rats. Lastly there needs to be a consideration put towards the size of plate that the subject will run on.

The spring blade is essentially a strip of material that is fixed with a transducer that is connected to the other leg with a span. There are in total two separate structures that consist of two legs each which are affixed with 2 transducer each. A platform is bonded to the crossbeam of both legs and is the area that is meant to be flush with the running track where the paws of the rat will make contact. This particular design is different from the original design proposed by Heglund by being composed of a box in the leg to create 2 spring blades per leg. The intention of this design choice at first was to allow for the use of phototransistors and LED as the way to measure displacement in from the force-plate but due to difficulties in the implementation of this particular sensor type the idea was disregarded and strain gauges were used. The design was kept though because the calculation had already been done and the parts had been made so it made sense to keep the design that essentially had 2 spring blades.

To determine whether or not the particular geometry of the force plate is correctly determined there needs to be a way to calculate the critical measurements when a force is applied to the platform resting on the legs. Necessary measurements would include the internal stress in the spring blade, the maximum strain, the natural frequency, and of course the dimensions of the geometry. There were clear limitations that needed to be satisfied by when preforming calculations to determine if a particular solution was appropriate to use and these limitation were discussed in the evaluation section of the Senior Design document. The equations necessary to find these values were the following:

Stress	$\frac{Mc}{I}$
Strain	$\frac{Mc}{IE}$
Frequency	$\frac{0.5}{\sqrt{y'}}$
Displacement	$\frac{FL^3}{3EI}$

Table 1: Necessary Equations

The maximum value for the force is 4.9 N so all calculations would assume this as the force and the length would be half the distance of the length of the box as outlined in the design from the original design that uses 2 cantileavered beams bonded at the center to model the spring blade(Heglund, n.d.). The preceding equations are the equations that are used to calculate the structural stresses in the spring blades that will be used to determine the forces applied to the force plate.

3.2.2 Material Selection

The material used to construct the force-plate was also an object of consideration when constructing the force plate. While most companies constructing force plates are using thin sheets of metal to create the platforms that will deflect or hold the sensors ours uses ABS 3D printed plastic. The force plate was constructed using acrylonitrile butadiene styrene plastic, otherwise known as ABS plastic due to its durability, rigidity, and ease of construction. This solution is opposed to using thin films of copper to construct the body of the force-plate spring blades because even though they have high natural frequencies, they are prone to difficult bonding procedures with strain gauges and they are difficult to manufacture for most labs.

The properties of ABS plastic are listed below and from these values we can begin to calculate what kind of strains, displacements, and stress values we can expect if a force-plate was to be created using this material.

Modulus of Elasticity (E) GPa	2.6
Yield Strength (S_{max}) Mpa	46
Minimum Thickness (μm)	16

Table 2: ABS Plastic Properties

These values made this material adequately strong enough to handle the loads imposed by the rats running gait and the minimum thicknesses we would need to use to construct the structures.

3.2.3 Dimensional Iteration

In order to choose the best measurements a program was created using the open source programming language "Julia" that allows for a terse syntax and compatibility across all operating systems without the need for paid licenses (Jeff Bezanson, n.d.). This code works by iterating through the different materials properties and the choices of dimensions for the spring blades and outputs the maximum stresses, strains, natural frequency, and the dimension and material of the spring blade.

This code can be found in the code listing section of the document as deflection.jl . The results of this is a .csv file that can be filtered to decide what values should be used based on ideal natural frequency values, strain, and material .

3.2.4 Final Design Values

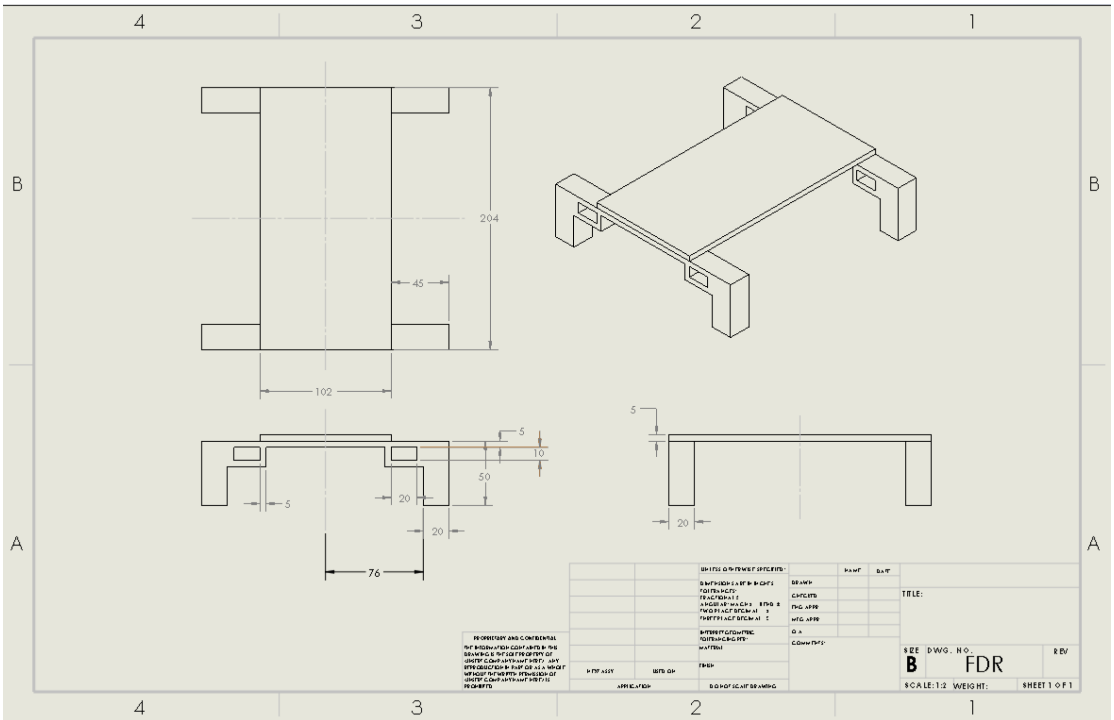

Figure 2: Final Force Plate Dimensions

3.2.5 Sensor Selection

The sensor type used for this particular force plate are strain gauges due to their ease of installation, their cost, and their success in past projects. In order to implement strain gauges in this project there will need to be consideration placed on how to bond them to the structure and connect them to a data acquisition board, how to process the data coming form the data acquisition board, and lastly where to source the strain gauges from.

The circuitry used to wire the strain gauges will be a half Whetstone-Bridge configuration with

3.3 Track Design

3.3.1 Structure Criteria

3.4 Experiment Design

3.4.1 Rodent Trainings

The rodents being used in the experiments are young adult female rats. Prior to introducing the rats to the experimental environment, they were introduced to human handling. Prior to having the ability to handle the rats, handlers must go through orientations, and complete online training sessions in order to familiarize themselves with laboratory procedures and protocols. The protocol for human handling follows strict adherence to safety rules within the animal housing facility. The rules in animal housing facility require the usage of shoe covers, full-body garments, gloves, facemasks, and hair nets. The purpose for such a strict dress code is to limit the transmission of any potentially transmittable diseases that could transfer to the caretaker's clothing. Following proper protocol to cover garments, the caretaker/handler can approach the rat cages. Each cage houses two rats. Once the rat cage is lifted away from the storage cart and placed on that table in animal housing facility, the outer lid of the cage may be removed. Following the removal of the lid, the first step to take is to reverse the direction in which the water bottle is facing; this eliminates the possibility of the rats in the cage to be drenched with water in any further transportation of the cages, and additionally, this allows for the internal cage lid, which houses the animal food and water to be lifted giving access to the rats within. In the case of transporting the rats from the animal housing facility to the animal testing facility, at this point after having removing the water bottle's nozzle from the downward position into the upward, the handler may replace the outer lid and proceed to carry the cage out in a bin large enough to store the entire cage as well as after completely undressing from the animal housing facility protocol rules; however, in order to handle the rats in the animal housing facility, the internal lid can be extracted off of the cage, this allows the handler to be exposed to the rats.

Now that the handler has access to hold the rats, a few certain steps must be take. First, be sure that all clothing garments are completely covering regions where you plan to hold the rat. Typically, when holding a rat, the chest area, as well as the arm area is used. Secondly, be sure that the door to the room in which you are handling rats is completely closed. This action is taken as a preventative measure in case the rat manages to escape the open top cage or the grip of the handler. Lastly, be aware of your surroundings. This prevents harm that could potentially hurt the rodent while in the care of the handler. Once these precautions have been made, the handler is ready to care for the rat. In order to lift the rat out of the cage, be sure that when the handler's hand is approaching the rat that the rat is not cornered; rats do not prefer being cornered, this triggers defensive mechanisms. Next be sure to approach the rat with confidence and firmness, however not so firm that the grip of the handler can cause harm to the rat. Once the handler has adjusted their grip, note that it is important to give support to the hind limbs of the rodent as you transfer the rat from the cage into your arms. To accomplish this action, be sure to grab the rat from the rear end and fully support the hind limbs with the palm of the grabbing hand. Once the rat is firmly in the grip of the handler, cradle the rat like a baby. be sure to provide support to the hind limbs of the rats as this reassures them that they are in caring hands. As you cradle the rat, be sure to have the hand which was used to lift the rat relaxed around the rat along it's spine. Additionally, with that same hand, comfort the rat by using one finger to gently run the region right around the rat's neck in the posterior region. In the event that the rat begins to squirm, replace the rat in the cage. In the early stages of animal handling, the rats require an acclimation period to adjust to the notion of them being grabbed and then set down. Once the handler has gained experience with handling the rats in the housing environment, the rats can be transported as mentioned above to the animal testing facility.

Having understood proper handling protocol, training the rodents can begin in the animal testing facility. The rats used were trained to run on a treadmill enclosed by a custom plexi-glass construction and were rewarded with Reese's Puffs as treats. After each run, the rat was rewarded with 2-3 Reese's Puffs. Over the course of several weeks, the time intervals of running versus resting were increased for the 5 different running speeds. The speeds at which the rats were trained at were measured in centimeters per second (cm/sec) and were the following:

- 16 cm/sec

- 20 cm/sec
- 24 cm/sec
- 28 cm/sec
- 32 cm/sec

For purposes to describe the training protocol, the protocol for the control rats is referred to ahead. For the first week of training, the rats were run at each speed for approximately 30 seconds to one minute (This time interval was increased throughout the week, with trainings being three times per week). The following week, the rats ranged from a 2-3 minute mark to a 5 minute mark by the end of the week. Each rest period in between all of the speed runs were approximately 2 minutes in the first week but since then were no longer than 1 minute. In the third week, the rats were trained at each speed for exactly 5 minutes and with 1 minute rest periods in between speeds. In the fourth week, the control rats had a hemi-section surgery performed through which they lost motor function to their right hind limb. For recovery purposes, the control rats were simply weighed and fed rewards in the animal housing facility during the sixth week. After a week of recovery, the rats were brought back to the animal testing facility and trainings were restarted at 5 minutes per speed with 1 minute rest in between. This was done fore 2 more weeks while every week post SCI, motion capture was used to analyze the gait of the impaired rats.

3.4.2 Control Data

3.4.3 Surgically Modified Data

3.4.4 DREADDs Data

4 Evaluation

5 Cost

6 Schedule

7 Summary & Future Work

8 Code Listings

Listing 1: deflection.jl

```
#!/bin/julia/

## Formulas To Calculate Values
#Formula to Calculate Moment of Inertia
MoI(b,h)=(1/12)*b*h^3
#Formula to calculate max deltalection
delta(F,L,E,I)=F*L^3/(E*3I)
#Forumula to calucate max Stress
S(M,I,c)=(M*c)/(I)
#Forumula to calculate the Natural frequency omegao
omega(y)=.5/sqrt(y)
#Forumula to calculate Strain
epsilon(M,c,I,E) = (M*c)/(I*E)

g=9.81; # m/s^2
#Plate Dimensions
#PexiGlas Plate
width=.204
base=.102;
t=.005;
rhopg=1.18*1000;#Density of PexiGlas in kg/m^3
Wp=base*width*t*rhopg; # weight of the PexiGlas plate

#Suspended Portion of Force Plate

#Youngs Modulus and Yield Strngths of different Materials
Names=["Digital ABS","Rigid Opeque", "Brass"];
YoungsMod=[2.6e9,2e9, 97.6e9];
Sy=[55e6,50e6,135e6];
rhomat=[1.17,1.17,8.7]*1e3;

# Dimensions to iterate over
l=linspace(0.5e-3,3e-2);
w=l;
h=linspace(160e-6,.5e-2);
f=open("dims.csv","w");
write(f,"Material Name, Length, Width, Thickness,Max Displacement,Natural
    Frequency,Max Strain\n")
for i=1:length(l)
  L=l[i]
  W=L
  for k=1:length(h)
    H=h[k]
    c=H/2;
    I=MoI(W,H);
      for x=1:length(YoungsMod)
        E=YoungsMod[x]
        Syield=Sy[x]
        rho=rhomat[x]
        Name=Names[x]
        Pmat=base*width*H*W/2*rho+Wp/4
        deltao=delta(Pmat,L,E,I)
        omegan=omega(deltao)
        if omegan>100
          P=Pmat+4.9;
          deltamax=delta(P,L,E,I)
          Smax=S(P*L/2,I,H/2)
          strain= (P*L/2,H/2,I,E)
          if Smax<Syield
```

```
            write(f,"$Name,$L,$W,$H,$deltamax,$omegan,$strain\n")
          end
        end
      end
    end
  end
close(f)
```

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