Mouse Sleep Deprivation Device

Biomedical Engineering Design 301
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Abstract

Dr. Rama Maganti is currently researching the effects of sleep deprivation in epileptic mice and how this impacts abilities to learn and retain memory. To do so, he is currently using an industry standard sleep deprivation cage which features a flexible plastic propeller on a circular motor which rotates and delivers stimulus to keep the mouse awake. However, the device itself is rather fragile and expensive, and the accompanying software is very limited in terms of how the cage can be programmed to operate. Thus, our design team has been tasked with developing a new and more complex program and to possibly redesign the cage if need be. Between three design alternatives, our design team has decided to improve upon the propeller concept in addition to developing software which will allow for far greater degrees of control of the device during experimental trials.

Table of Contents

Problem Statement

Dr. Rama Maganti intends to study the effects of sleep deprivation in pubertal and epileptic mice and how this affects learning and memory. To accomplish this research, a device is needed that can awaken mice as they are beginning to fall asleep. The device should be easily programmable by a user and should monitor the mice, deliver a non-painful stimulus to awaken the mice, and allow arbitrary control of this function.

More specifically, this device should be able to keep mice awake for 6 hours to 2 weeks at a time. This device must ensure that the mice do not fall asleep even for 30 seconds, should be able to be taken apart to be cleaned, should allow mouse access to food, and should not injure the mouse in any way. The device should respond to the mice trying to fall asleep, should be able to be programmable by the user for experiment customization and any programs created should be able to be run on multiple mouse cages at the same time.

Background

Dr. Rama Maganti is a professor studying the relationship between sleep loss, epilepsy, and memory. Epilepsy stems from a series of neurophysiological disorders and is characterized by spontaneous seizures. They encompass a very wide range of seizure types and causes, but are united by the common attribute of synchronous neuronal activity that is abnormal or excessive (1).

It has been seen in past research that there is a physiological relationship between sleep loss and a decrease in ability to code and store long-term memory (2,3,4). When individuals learn, long-term storage of this memory occurs when the brain "rehearses" this information by stimulating and re-stimulating neural pathways associated with that memory in the hippocampus. Typically, the brain accomplishes these actions during sleep (2). It has been suggested in scientific literature that the reverse correlation between sleep and memory may be caused by a number of different factors, including deficiencies in microtubule proteins, adenosine buildup in the hippocampus, or in increase in oxidative stress in the brain. However, all of these three have been shown to have a detrimental effect on memory in mice or rats (2,3,4). Therefore, the exact mechanism for this is not well understood.

Additionally, a good deal of research has been done on the relationship between sleep loss and epilepsy. An increase in epileptic activity has been shown to occur with sleep deprivation in both mice and humans, but scientists question the mechanism for this as well (5). In fact, researchers are even unsure whether or not the increase in seizures is the cause or result of sleep deprivation (5). However, it has been seen that sleep loss potentiates damage in brain cells that are already hurt by epilepsy (6). Therefore, it is important to better understand how these three mechanisms are related in order to better treat individuals who experience problems associated with memory and epilepsy.

Dr. Rama Maganti and his research assistant Eli Wallace are investigating this relationship in order to develop a better understanding of its bimolecular origins as well as to develop a quantifiable model for how and to what degree this occurs in mice. The long-term objectives of their research are to apply any correlations between sleep loss and epilepsy found in mice and apply these relations to treating and handling human individuals.

To meet these goals, mice that are predisposed to epileptic seizures are bred for experimentation. They are then subjected to a certain degree of sleep deprivation, and the mice's change in behavior with regard to memory driven tasks as well as chemical changes in their brain is observed. The data is then compared with that of sleep deprived non-epileptic mice along with their non-sleep deprived peers. The mice, which have sleep cycles as short as 30 – 90 seconds, are deprived of sleep for 6 hours to up to 3 weeks at a time using a physical stimulus to awaken them.

Dr. Maganti and Eli Wallace currently use a "smart" rodent cage for their experiments from a product manufacturer named AfaSci (7). This product allows tracking of position, movement, active time, and speed of the mice. Additionally, they have bought several add-ons for his products that can induce sleep deprivation in the experimental mice by means of a small, flexible plastic propeller that turns with the frequency desired by the consumer. The propeller is flexible enough so that it will not hurt the mouse but will provide a stimulus to wake it up; however this product has several problems. First, the materials it is made out of are not very sturdy or durable, and it must be handled very carefully. Secondly, the program for the control of the propeller is very limited, and must be updated anytime the user wants to change the frequency of the motion of the propeller. Finally, the mechanism to track the movement of the mice is not very precise. Therefore, they desire a system that completes the same functions as his current product but with greater versatility and durability.

Product Specifications

The final product must contain stable housing for one mouse. This housing must be able to provide access to food and water and must be able to fit in a 20 by 35 cm area. The product should have an intuitive user interface for programing speed, frequency, and duration of mouse stimulus and must be able to operate continuously for up to two weeks. The client has also stated that the device should ideally have a mechanism for monitoring the mice and deliver a stimulus when the mice are showing signs of sleep. Additionally, the device should be able to operate and store experimental data without being connected to a computer. However, the client has said that these last two requirements are not crucial to the success of the device.

The device will be used on a weekly or semi-weekly basis, and will house a mouse weighing 25-50 g. Because of the intended use of the device, all materials and processes used must be able to be approved by the IACUC and the cage must be transparent in order to be able to see the mouse inside it. Additionally, materials should be chosen to fit the following specifications: first, the device will be operating continuously for up to 2 weeks at a time, and therefore considerations must be made so that the device does not overheat. Second, the stimulus delivered by the device, due to the length of the experiment, may be delivered several hundred to several thousand times during a single use (720-40,000, or, once every thirty seconds for 6 hours up to 2 weeks), and this must be considered when choosing materials for a motor and circuitry. Third, materials may come into contact with mice bodily fluids, and this must be considered when choosing materials. Finally, the device should be able to conduct experiments on a weekly or semi-weekly basis for at least two years.

Mice should not be able to sleep at all over the course of the experiment. While scientific literature has shown that 100% sleep prevention is nearly impossible (8), the device should be able to prevent sleep in mice at least 97% of the time in order for experiments to be considered effective. As mice sleep cycles can be as short as 60 seconds in length, and the delivered stimulus must not injure the mouse, this must be taken into consideration when designing the software as well as selecting materials for the stimulus and determining stimulus strength.

Finally the client has requested that the device be able to be autoclaved between uses. This means that any non-removable components of the device must be able to withstand 82 degrees C for 5 minutes at a time.

Existing Products

Forced exercise/walking wheel

The forced exercise and walking wheel device consists of a bed with wheel tracks that spin all of the wheels on the bed simultaneously [9] (figure 1). The bed itself costs $3,600 dollars and each walking wheel costs $505. Underneath are steel waste pans to collect waste from the mice. There are swing hatches on each cage for handling and a LCD interface where the user can set the exercise, walking speed, exercise time, rest time, and number of cycles. Also there is a built in USB to connect to the computer. This particular device works with a program that the company designed called AWM Activity Software. The speed range is 1-28 m/min with a speed resolution of 0.5 m/min. The test time range is 0-24 hours or 0-99 cycles. Water support options for this particular device cost an additional $99 each. The wheels have polycarbonate sides, with aluminum rungs [9]. The advantages of this device are: the LCD interface, the variables in the program, and the waste collection. The drawbacks of this device are that is only goes for the duration of a day, costs more than $8,000 total, and forcing the mice to do exercise had adverse effects on the research our clients are conducting.

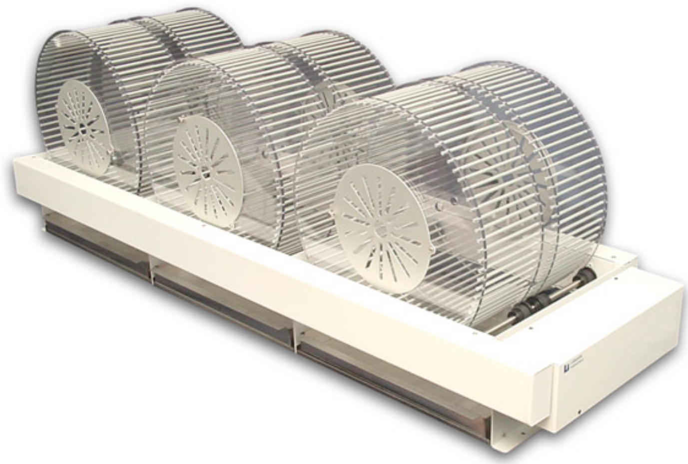

Figure 1 - Forced exercise/walking wheel existing product: A set of exercise wheels with waste collection trays below them for the purpose of sleep deprivation experimentation.
[9]

Sleep deprivation chamber

The sleep deprivation chamber utilizes a sliding bar on a track to keep the mice awake by forcing them to step over the bar (figure 2). There are several forms of this device that feature a sweeping horizontal bar. The product from the Lafayette Instrument company provides water and food support, an inter-cycle time of 15 seconds to 20 minutes, and needs a 28V DC power supply [10]. One of the other versions has speed, torque, and movement intervals variables in the program and the ability to detect the end of the cage and relay engages it in the other direction [11]. The cost for the Lafayette product is approximately $1,650, but according to their product manager the motor has become obsolete (it makes too much noise and didn't provide enough torque). These products have good support for physiological needs, provide a unique tactile stimulus, and have several programmable variables; however the cost, noise, and difficulty with cleaning and maintaining the motor and track are the less favorable aspects to this product.

Figure 2 - Sleep deprivation chamber existing product: A device which features a sliding bar which is swept across the bottom of the cage forcing the mice to walk over it. [10]

Rotating drum

The rotating drum model has a fixed interior wall and a bottom plate that the rodent rests on which rotates (figure 3). While this product has been used with rats and has been shown to be extremely effective, it has yet to be tested for use with mice [12]. Part of this is because of the research format. Speed and directional variability gradually increases to compensate for increasing sleep pressures as the experiment progresses. Sleep state is detected by infrared sensors, along with EEG and EMG. Part of the experiment in which this product was used was intended to research social interaction and how it connects to sleep deprivation, so in between the two compartments are drilled thirteen holes. The drum wall is made of plexiglass and the rotating plate is aluminum [12]. Water and food are provided via tubes in the central wall. While this device has proven very effective, it has not yet been tested on a size scale relative to mice and is very mechanically complex and difficult to fabricate.

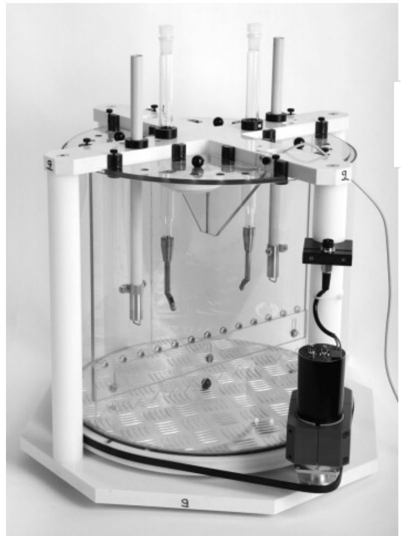

Figure 3 - Rotating drum existing product: A barrel shaped cage with a rotating bottom to entice movement and ensure deprivation of sleep. This model was also developed for used with two rodents for experiments separated to observe effects of social interaction. [12]

Propeller (existing)

The final existing product is this propeller-based design being produced by Pinnacle Technology Inc. which is priced for approximately $4,000-$7,000 depending on the model (figure 4). The tactile stimulus for keeping the mice awake is the propeller that spins around, mimicking gentle handling. It is possible to yoke multiple cages together to form a system and each unit can stand alone or connect to a computer directly. Scheduling can be done via the LCD screen and it features an EEG/EMG recording system. The bar has a variable spin rate, from 5-15 rpm, and can change direction. This product also accommodates physiological needs: bedding, food, and water. The cage itself is 10 inches in diameter. The exorbitant cost is the crippling disadvantage to the possible use of this design.

Figure 4 - Propeller existing product: A propeller raised from the bottom of the cage spins gently in a rotational fashion and delivers stimulus to waken the mouse. While effective, the product is very expensive. [13]

System Process

The start of the process for a sleep deprivation device begins with user input. The user can define the program that they desire to run with preferences ranging from frequency of to duration of the experiment. The computer that defines the program connects with a microcontroller. The microcontroller is the connection between the computer and the circuit itself. Then circuit takes the signal from the microcontroller and implements it into the motor. And lastly, the motor then operates the cage specific components and wakes the mouse in the cage according to the program that was set up by the user.

The client's sensory devices are connected to the mouse and perform all the necessary data collection. The mouse's state of sleep and EEG are recorded. This sensory data is then sent to a computer or in cache-memory stored independently within the cage. With either data storage scenario, the data is eventually retrieved by a computer. Then the computer accepts the data from the memory, sorts it, displays it in the program, and converts it into a universal data type to be accessed by other programs for the client (figure 5).

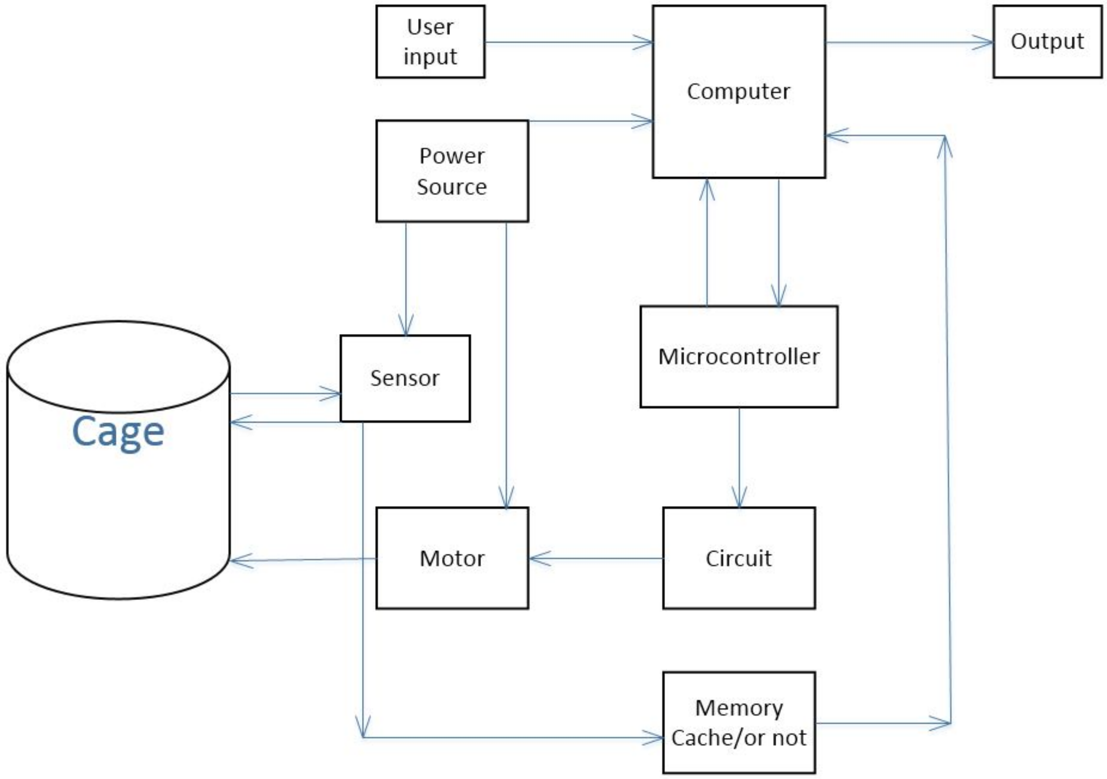

Figure 5 - System Block Diagram: Starting from user input into the program, the computer sends the user's specified program to the microcontroller which translates this information and sends it to the motor via the circuit. The client's sensor devices are independently managed outside the scope of this process. All sensory data is however sent to a computer or a cache-memory for later retrieval.

Cage Design Alternatives

Slide Bar

The slide bar design alternative dimensions are identical to those of the cages the client is currently using: 8 inches wide, 14 inches long, and 10 inches tall (figure 6). It features a bar along a track with a motor that slides it back and forth along the bottom of the cage. The motor and track are housed together in a separate section on top of the cage. The slide bar operates by forcing the mouse to step over the bar, effectively waking it up. And ideally the slide bar would detect when it is close to the side of the cage and reverse directions.

There are three holes in the top of the cage. The smallest one, close to the edge, is for electronic cables running between the mice and computer. And the purpose of the two larger holes is for the dispensing of food and water to the mouse. In addition, the motor/slide bar complex can be removed for cleaning and the rest of the parts can be autoclaved. This device has the advantage in that it is fairly sanitary and has been proven effective in preventing REM sleep [11]. Potential complications that could arise with this device are closely related with the complex mechanics of the engine and the track the sliding bar is on.

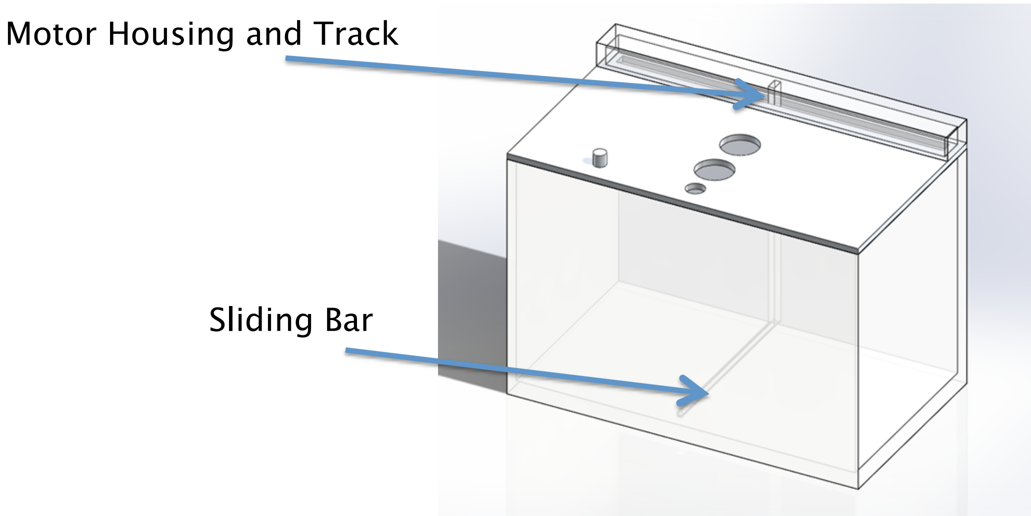

Figure 6 - Slide Bar Alternative: The slide bar design features a bar which sweeps from one end of the cage to the other along a track, forcing the mouse to react and walk over it.

Propeller

The propeller design features a round cage with a diameter of 6 inches (figure 7). This allows the mouse the maximum living area while still fitting snugly in the housing container. The cage is approximately 8 inches tall, which prevents the mouse from escaping. Halfway up the cage there is a cut in the side of the cage to dispense food and water into the cage for the mouse. The key feature of this design is a propeller protruding from the bottom of the cage which is mounted onto a spinning bar. This bar is centered on top of a rotating motor that controls the speed and direction of the propeller. The propeller radius of 2.9 in. was selected to ensure that the mouse would be disturbed by the propeller, but the propeller would not have problems with being flush with the cage. And propeller itself is made of soft, flexible plastic material, so the mouse would not be harmed when contacted by the propeller.

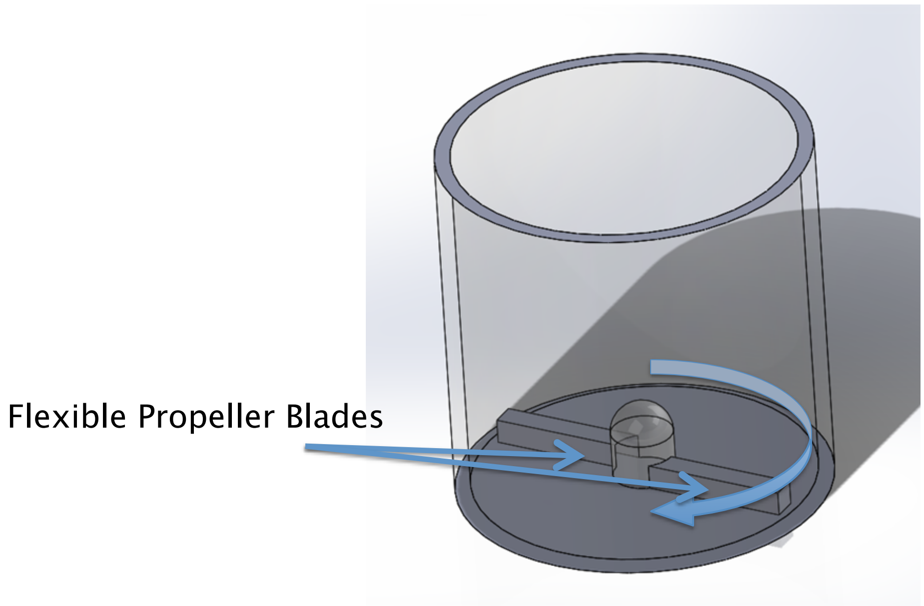

Figure 7 – Propeller Alternative: The propeller design consists of two flexible propeller blades attached to a rotating motor which are capable of rotating clockwise or counter clockwise. Contact with the propeller blades is the stimulus delivered to wake the mouse in this design.

Platform

Our platform design consists of a circular cage 6 inches in diameter, a circular platform suspended above the bottom of the cage by its connection to the motor, and a shallow pool of water (figure 8). This design wakes the mouse through the partial rotation of its platform. This rotation causes two things: 1) It creates a change in the incline of the platform that forces the mouse to readjust its center of gravity and 2) It forces the mouse to avoid the parts of the platform that are becoming submerged due to rotation. Studies employing EEG, EMG, and theta activity as methods of sleep detection have reported effective reductions in NREM sleep (96%) and that disk rotation occupied around 16% of the total trial duration in such cases [14]. Food and water are provided from above along the axis of the rotation for the platform to allow for ease of access during any time throughout the duration of the trial.

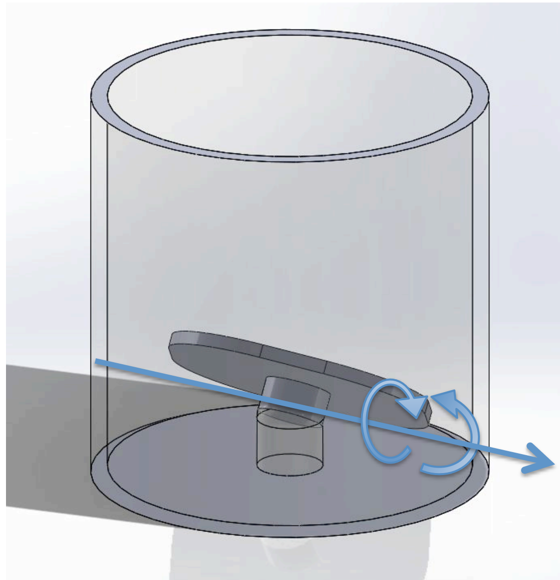

Figure 8 – Platform Alternative: The platform design features a platform which partially rotates in either direction along an axis above a shallow pool of water which partially submerges one side into the water. The incline of the platform and the evasion of the submerged portion are the stimuli to prevent the mouse for sleeping in this design.

Design Matrix Analysis

Design Criteria	Weight	Design Alternative		
		Slide Bar	Propeller	Platform
Ability to Wake Mouse	20	15	12	15
Ability to Implement Software	15	10	10	10
Ability to Implement Circuit	15	7	15	5
Ease of Operation	15	10	15	5
Ease of IACUC Approval	10	10	10	7
Feasibility	10	6	8	4
Ease of Sterilization	5	3	5	3
Ease of Producing >1	5	3	5	4
Cost	5	3	5	3
	Total			
	100	67	85	56

Table 1 - Design matrix evaluating design alternatives: The propeller design alternative received the highest rankings in nearly all categories due to the relative simplicity of its design whereas the slide bar and platform design alternatives had problems associated with complexity and waterproofing respectively

The designs were evaluated according to a wide variety of categories because there are many components that contribute to the success of the prototype. These components include: the ability of each design to wake the mouse, the compatibility of the design with the intended software, the compatibility of the design with the intended hardware, ease of everyday operations of the design, the ease with which the design will receive IACUC approval, the feasibility of the design, the ease of sterilization using autoclave of the design, the ease of production of the design, and the cost of the design.

The "ability to wake mouse" component of the design is the most important parameter for evaluation because this is the primary function of the design, and without it, the device would not be useful. All three waking mechanisms in these designs have been studied scientifically and have been shown to be effective. All designs have been seen in scientific literature to be able to wake the mouse to an effective degree to be used for research but the propeller design has been seen to be the least effective in doing so: the flowerpot technique (from which the tilting platform is derived) has been seen to prevent sleep at least 90.4% of the time, and the addition of the tilting platform onto the current technique will likely improve this (15). The sliding bar design has been seen to prevent sleep at least 95.5% of the time in sleep fragmentation studies, and this will also likely improve in total sleep deprivation (16). Due to the varying flexibility of the propeller over its length, we can infer that the propeller will be less effective than either of these two designs. However, the propeller design is still an industry standard, and the torque applied can be varied with the plastic chosen as well as other factors and therefore receives a relatively high ranking.

The compatibility of the design with the software and the circuitry was considered the next most important parameter, because compatibility in these two areas was vital for the successful operation of the design. All three designs received a high score for compatibility with the program as the program would be universal to all three. The differences lie in the implementation of circuitry for each design. For this, the propeller design was given the highest ranking because it simply requires a circular motor, and overall it is rather simple terms of its mechanical components. By contrast, the platform design needs motors that can go in multiple directions in order to tip the platform in varying directions and needs to be designed considering the aquatic environment surrounding the platform. Similarly, the sliding beam design needs more complex hardware and tracks for the design to be successful, as the beam needs to travel the length of the cage.

Ease of everyday operation was also weighted heavily as it is important for the day-to-day operations of the device such as restocking food and water, cleaning the cage, and resupplying of the bedding to be handled without the device breaking down or encountering problems. The propeller device was given the highest ranking due to the simplicity of its design; the discrete propeller size and lack of a great deal of complex mechanics cuts down on the number of aspects of the device which could be likely to degrade and break down over time. This makes cleaning and the potential for restocking broken components relatively easy. However, the platform design would be rather difficult to clean because of the standing water in the device. The concern of any watertight sealing leaks leading to the circuitry damage is also another problem which could easily arise from improper fabrication. Finally, the slide bar design has many components which could break down in day-to-day operation and the large size of the circuitry makes cleaning while still running the experiment difficult.

The ability of the design to meet IACUC sanctions and the feasibility of construction of the design were given the next highest rankings. All designs will meet basic standards of care for mice and will therefore meet IACUC sanctions. However, the water aspect of the platform cage will inherently introduce further sanitary concerns and will therefore make it harder for this design to receive this approval, while the other designs have no foreseeable complications for IACUC approval. Feasibility of construction is important considering the time constraints placed on the project, but should not be a dominating factor in choosing a design. Similar rationale was applied to this category as to some of the other categories in ranking the designs: as the platform design needs extensive waterproofing, it was given a low feasibility ranking. Extensive waterproofing and ensuring that these watertight seals do not leak poses problems that make the design less feasible. The slide bar was also given somewhat of a low feasibility ranking due to the complexity of its extensive circuitry.

Finally, ease of sterilization, ease of larger scale production, and cost were given the lowest weighting. Sterilization and larger scale production were given this lower ranking because they were aspects that were desired by the client but were not crucial in comparison to the product's performance. Additionally, the client desires eight prototypes for use. However, it was decided that these could, if necessary, be made at a future date. Cost was given a low weighting because the budget is much larger than the foreseeable expenses of this project. The platform and sliding beam design received lower ratings in all three of these categories due to their respective design complexities associated with waterproofing and more extensive mechanics respectively.

The propeller design received the highest total ranking and received the highest ranking in every category except for the ability to wake the mouse. The slide bar alternative received the next highest total ranking with deficiencies resulting from its complex circuitry. Finally, the platform design received the lowest ranking due to complications arising from the inclusion of water into the design. Therefore, the team will proceed forward using the propeller design as a final design.

Final Design

Component – Software

The final device will contain a software program that allows the user to control the frequency, duration, and directionality of the propeller in the cage. The software will be programmed in LabVIEW, and will contain a user interface. In this user interface, the user will be able to input the total time for the project and, for up to 10 different sequences associated with the project, input the following: the start time for the sequence, the end time for the sequence, how often the stimuli should be delivered, and for how long of a time the stimuli should run for during each delivery. The ability to run up to 10 different sequences will allow the user to be able to have varying speeds and frequencies of the stimulus as needed over the length of the experiment, something that is lacking in the current device. For example, the user may desire, for the first four hours of the experiment to only deliver a stimulus once every half an hour to simply acclimate the mouse to its surroundings, but later in the experiment they may desire to deliver a stimulus once every thirty seconds because the mouse is very tired and constantly attempting to fall asleep. This feature allows the user to input these variables only once without having to re-input the desired features for each change.

The program will create a timer from the total time of the experiment specified by the user. Then, if the time left of the experiment is greater than zero, the program will check to see if there is another sequence queued by the user. If so, it will create a timer based on the start time and end time for that sequence specified by the user, and will begin a countdown, turning the motor on and off with the frequency specified by the user. When the countdown reaches zero, the program will then check against the total time of the experiment and then for another queued sequence. And then the process will repeat. The software will record the times and frequencies for later analysis. Finally, the software will be connected to the rest of the circuitry through a microcontroller, whose brand and model have yet to be determined.

Circuitry

The final design would feature circuitry to connect the different components that need power together. This includes the eventual connection to a microcontroller from the computer. The microcontroller will then be connected via the circuit to the power source, sensors, and motor. The electronic connection between the sensor, memory, and back to the computer (as can be seen in the project flow diagram) is also part of the circuit. Based on current designs and research, the circuit board often features a microcontroller of sorts and, based on the software, a PIC microcontroller would be the best for the design. When collecting data, outside factors can affect the quality of the signal and result in noise. There can be shot noise, where there are random fluctuations of the electric current, thermal noise (Johnson or Nyquist noise) from random thermal motion of charge carriers, 1/f noise (flicker) where the signal consistently falls into the higher frequencies, noise from interference from unwanted signals, accidental grounding, and noise from physical interactions between wires and nearby objects (especially ones with electricity of their own) [17]. The circuit will include several filters, the exact nature of which is part of the future work. A serious consideration for the circuit is the requirement that there be no wires that can be accessed by the mice to chew on.

Cage design

As of now, our plans are that the final cage design will be constructed from transparent, circular polycarbonate tubing with an outer diameter of 6” and an inner diameter of 5.75” and stand approximately 8” tall. The base will likely machined from a sheet of polycarbonate and affixed to the tubing by some form of adhesive or other consideration. Polycarbonate was chosen due to its adherence to the requirements of being clear and transparent, so the mouse can be observed from outside the cage. Another determining factor was polycarbonate’s heat deflection temperature satisfying Autoclaving requirements [18].

Our final design will feature a propeller system analogous to the propeller design alternative. The material still has yet to be decided, but will be some derivative of a soft flexible plastic which will likely have to be sanitized separately as opposed to autoclaving. Food and water will be provided in a similar fashion from pre-cut wholes at an accessible distance from the bottom of the cage for the mouse. Also, the propeller system will eventually be separable from the cage itself in an effort ensure the prototype can be deprived of all its electronic components and autoclaved for ease of sanitization.

One crucial aspect of the final design of the cage that links the various components of this project is the propeller. This seemingly small detail is extremely important in the overall design because it must be sturdy enough and provide enough force to wake the mouse up on a consistent basis, but must not injure the mouse upon repeated impacts. Therefore, one of the first steps taken in the final design refinement was the calculation of the force required to move a mouse, as described below:

First, it should be considered that the force required to move a mouse that is sleeping will be approximately equal to the friction force of that mouse in contact with the base of the device.

Second, that force will be equal to the coefficient of friction between the mouse and the plastic multiplied by the mass of the mouse and the gravitational acceleration.

Next, the torque associated with a rigid propeller will be equal to the force needed to move the mouse multiplied by the length of the propeller.

The angular velocity of the propeller for an average rotational speed seen in current devices is calculated.

Finally, the power needed to turn this propeller at this frequency is shown above. This calculation, though not extremely accurate, gives us a baseline idea of the torque needed to be applied to the propeller to move the mouse as well as the power needed to be supplied to the motor. However, these calculations do not take into account the flexibility of the propeller, any mouse resistance to movement and use a low coefficient of friction that may not be accurate considering the mouse feet are in contact with the plastic surface (19).

Moving forward, it will be important to keep these equations in mind, as they play a role in the selection of materials for the propeller as well as specifics associated with the motor qualities and circuit design. It will be important not to deviate a great deal from the numerical values found here and in more accurate calculations in order to ensure functionality of the device without causing permanent harm to the animal.

Budget

As mentioned previously, the budget for this project will likely not be exceeded by the projected costs for this design. At present we estimate roughly $150 for the circuitry components of the design which would include things such as: wiring, an Arduino microcontroller, motors, etc. While not the intended material, research for acrylic tubing of the desired measurements for eight cages priced at around $180. Given the higher quality of polycarbonate we estimate currently anywhere roughly from $400-$500 (including sheets which will be machined for to size for the bases) for eight cages worth of material. Naturally, these are rough estimates, and the team will strive to be as economical as possible.

Ethics and Animal Welfare

This design project contains several elements that could be potentially considered ethically objectionable if not addressed properly. The following presents a series of ethical considerations that must be taken into account considering that this product will be used with a live animal.

First, the inclusion of a live animal into scientific research poses a need to care and maintain the basic level of health of the animal. All animals used in this research must be provided food and water in order to not create unwanted stress for the animal, for both humane and research reasons. Additionally, the animal habitat must be sanitary. Therefore, the cage that the animal lives in must be properly sanitized between uses as well as cleaned and the animal bedding changed on a regular basis. Proper sanitation will prevent animal odors and disease, and this, in turn, will prevent unwanted stress in the animal as well as provide a humane living place for it. Considerations for animal care according to the Guide for the Care of Research Animals, such as the size of the device and ventilation associated with the needs of the animal, have been taken into consideration in the device design. Additionally, these factors have been considered in the design matrix for the selection of the final design.

Secondly, this research involves the deprivation of sleep to an animal. This is, in and of itself, a seemingly ethically questionable research method. However, an animal research regulatory board must approve experimental protocols in order to begin experimentation. Our clients have extensive training in animal handling and their experimental protocols have been approved and are fully within the limits outlined by the IACUC. This fact notwithstanding, it is important for the team to consider any changes in the ethicality of the experiment posed by the use of this device. Therefore, in the final design, it will be important to minimize any pain or discomfort this design may inflict on the mouse, and, because of this importance, this consideration was one of the first steps taken in the refinement of the final design: the minimum torque required to move a mouse has been calculated and the team will take steps to ensure that the design does not implement a torque that is much greater than this.

In the further refinement of the final design as well as the building of the prototype, there are many other important considerations to take into account when designing a device that comes into contact with animals and should not increase this animals' distress any more than necessary. For example, steps will be taken in order to ensure that the animals do not have access to hazardous circuitry components, and care will be taken to ensure that there are no sharp edges in the final design that could harm the mouse. It will be paramount to continuously consider the Care of Research Animals Guide and maintain open communication with the UW-RARC upon continuation of this design (20,21,22)

Future Work

For the remaining portion of the semester, we will be constructing and coding the aforementioned elements. The primary goals for the semester are to create a durable prototype that can withstand the sterilizations methods desired by the client, as well as creating a software system and accompanying circuitry that can induce sleep deprivation in mice. Within each of these goals there are many smaller steps that must be taken that include, but are not limited to the following:

First, for the mouse housing itself, materials must be selected and purchased. The design must be finalized to reflect the placement of holes for the delivering food and water to the mouse, as well as the placement of holes to incorporate EEG leads and motor circuitry. Finally, the materials for the propeller will be selected and ordered after considering torque and force applied with different propeller material parts. Then the design will then be fabricated.

Second, for the circuitry, a microcontroller and motor must be researched and selected. Other circuit parts including wiring, resistors, and any additional hardware must also be selected and ordered. The circuit must be designed, tested, and built, and finally interfaced with both the computer program and the mouse cage prototype.

Third, the software must be coded to reflect the client wishes and the program design outlined above. This will require assistance, and we plan to ask for help with this aspect of the project.

Additionally, the team will research and develop a recommendation for how to integrate a mechanism that monitors the mice being used and only delivers a stimulus when they are falling asleep. This was a feature that was initially requested by the client, but deemed to be too difficult of a problem for the scope of the semester and will only be primarily researched this semester.

The team will also research how to integrate a wireless memory and programming system into the mouse cage so that it does not need to be connected to a computer at all times. This was also a feature that was initially requested by the client but was deemed to be too great a challenge for the scope of the semester.

Finally, we plan to test the device this semester. First, we plan to test the durability of the product by subjecting it to varying stresses. Though this is not a primary function of the prototype, the durability of the current product was a primary complaint of the clients. Second, we plan to complete extensive testing of the software and circuitry associated with the prototype. We plan to test the accuracy and limits of the program associated with frequencies and durations of the running of the motor. Though this will have already been calculated in the design, we plan to test the torque of the motor, and ensure that the stimulus applied will not injure the mouse. We also plan to test the longevity of the circuitry and the software by leaving the prototype running for an extended period of time. Finally, we plan to test the feasibility of using our prototype for research purposes by incorporating a mouse into the prototype and observing how our device compares to the current devices in terms of mouse manipulation and ease of use. Additionally, in order to receive approval for this final phase of testing, the prototype will need to be approved by the IACUC.

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Appendix

A. PDS

Client Requirements:

- Stable housing for 1 mouse that provides access to food and water, fits within a 20 by 35 cm area and is able to deliver a stimulus that will awaken the mouse.
- Intuitive user interface for programing speed, frequency, and duration of mouse stimulus.
- Feedback system that can monitor the mouse and deliver a stimulus if the mouse is falling asleep
- System must be able to operate continuously for up to three weeks and should be able to operate and store mouse data without being connected to a computer
- System must be able to be sterilized between uses and should be able to withstand 82 degrees Celsius

Design Requirements:

1) Physical and Operational Characteristics

a. **Performance Requirements:** Device will be used on a weekly or semi-weekly basis and will house one mouse at a time. The mouse will weigh, on average, 25-90 g and can escape through an aperture of 6 cm^2. The stimulus delivered by the device will occur several hundred to several thousand times over the course of the experiment (720 - 60,000).

b. **Safety:** All materials and systems used in contact with mouse will be subject to IACUC regulations. Additionally, it is vital to note that any exposed electrical or chemical elements in the mouse housing are a safety hazard to the mice. Finally, since the device will be operating continuously for extended periods of time, careful consideration must be given to ensure that the device does not overheat.

c. **Accuracy and Reliability:** Mice should not be able to sleep at all during the course of the experiment (ideally the device should promote 100% sleep prevention). In practice the device will prevent sleep at least 97% of the time and will be able to do so over a period of up to 3 weeks.

d. **Life in Service:** Experiments using the device range from 6 hours to 3 weeks in length. Therefore the device should be able to deliver up to 60,000 stimuli over the course of one experiment (1 stimulus every 30 seconds for 21 days). Additionally, the product should be able to be used for at least 2 years.

e. **Shelf Life:** While storage of this device will occur in normal environmental conditions, materials and circuit components will be chosen such that no component of the device will degrade within the 2 year minimum life of the product.

f. **Operating Environment:** Corroding could result from mouse bodily fluids, and materials will be selected such that this does not occur.

g. **Ergonomics:** Any stimulus used should not cause lasting physiological or psychological damage to the mouse. Additionally, mouse housing must provide reasonable space for mouse bedding and general habitability in accordance with IACUC regulations.

h. **Size:** Housing must be able to fit in pre-existing 20 by 35 cm mouse cage.

i. **Weight:** Must be able to be carried easily by researcher (<4.5 kg) but must also not be able to be lifted by mouse to allow escape (>0.45 kg)

j. **Materials:** As stated previously, any materials used must follow IACUC guidelines. Additionally, materials should be able to withstand 82 degrees Celsius for 15-20 minutes. Finally, materials should not be able to be chewed through or degraded by mouse action.

k. **Aesthetics:** Mouse cage should be transparent.

2) **Production Characteristics**

a. **Quantity:** 8

b. **Target Product Cost:** $1000 for 8 mice sleep deprivations devices plus software and circuitry

3) **Miscellaneous**

a. **Animal Restrictions:** All materials used, sleep deprivation mechanisms, and cage conditions are subject to IACUC regulation because of the involvement of live mouse specimens.

b. **Client Specific Information:** Mice can sleep for as little as 30-90 seconds at a time. Even this much sleep is unacceptable. Therefore, the device must either be extremely precise in monitoring the mice or must be able to deliver a stimulus every 30 seconds for the duration of the experiment.

c. **Sterilization:** Materials will be sterilized between use and should be able to be autoclaved (withstand 82 degrees Celsius for 15-20 minutes)

d. **Competition:** Many rodent sleep deprivation devices are currently available in market. Examples of these can be found below. The aim of this project is to create a cage coupled with a software that is more widely applicable (is not product specific) and less expensive than current devices.

Appendix I: Links to current devices and IACUC guidelines:

Current Devices:
https://www.lafayetteneuroscience.com/product_list.asp?catid=120
www.limef.com/downloads/sleepscience_2009.pdf
www.limef.com/downloads/FV_2008.pdf
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982737/
www.sciencedirect.com.exproxy.library.wisc.edu/science/article/pii/50165027011000409
www.pinaclet.com/sleep-deprivation.html

IACUC Documents and Guidelines page:
http://www.iacuc.org/usa.htm