RODENT AUTOMATED TRAINING

Progress Report

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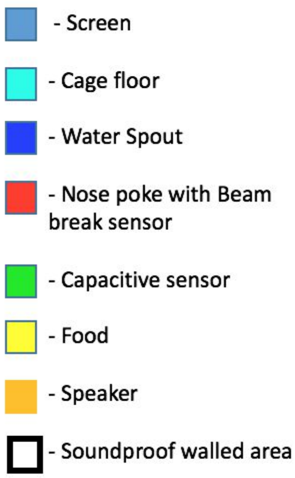
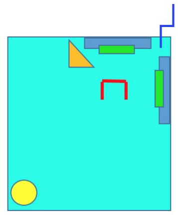
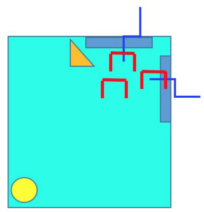
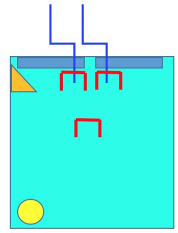
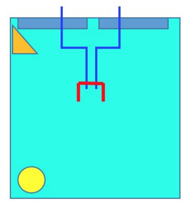
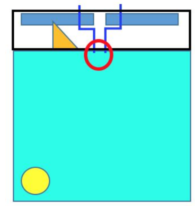
CHANGES FROM PRELIMINARY REPORT

The Rodent Automated Training (RAT) system is designed to automate rodent training for their performance of a forced-choice visual discrimination task. There are three main changes to the plan presented in the Preliminary Report. First, the beam break sensor is no longer its own project with an individualized schedule - it will be developed concurrently with the food and water delivery work, due to the RAT system's shift to a nose-poke water delivery system. Zoe will take the lead on the beam break portion, and the timeline for food and water will be extended by one week. Second, Sara will be assisting Zoe on the temperature and humidity project, which has shifted its focus from environmental control to prioritizing the monitoring of temperature and humidity. This project will still integrate environmental readings into the lab systems. Third, the possibility for rodent interaction during studies will be included with the modification of one of the cage walls.

DESIGN ALTERNATIVES

The RAT system is comprised of a cage with automated food delivery, water delivery triggered by a beam break sensor, two screens, automated light and sound control, rodent nose-pokes, and temperature and humidity monitoring. Several alternatives exists for each of these components, as well as their orientation within the cage. The diagrams included in Table 1 detail five potential cage layouts suited for the client's visual discrimination task. Designs 1 through 5 specify potential arrangements of all components excluding lights, shared wall, and temperature/humidity sensor; the location of the lights and sensor do not influence the RAT system's ability to train rodents for a visual discrimination task. The lighting and the temperature /humidity sensing mimics the cages already in use at the Hengen lab. The location of the shared wall between rodents also mimics what is already in use. A string of LED lights line the perimeter of the ceiling, and a temperature/humidity sensor is mounted on the ceiling.

Table 1: Diagrams of the different cage designs

Key	Designs 1 and 2	Designs 3 and 4	Design 5
	1.  2. 	3.  4. 	5. 

Design 1 includes two screens that form the upper right corner of the cage. This design includes capacitive sensors that the rodent will need to touch to indicate that it has made a choice between the images on the different screens. By touching the correct capacitive sensor, water will be available via the single water spout in this design. Additionally, this design has a nose poke with beam break sensors. In order to receive the reward, the rodent will need to stand in the nose poke area and break the beam for at least 2 seconds. This will ensure that the rodent is looking at the images from the same position each time it participates in training and not just touching the sensors at random to get water.

Design 2 includes the same screen setup as Design 1, as well as the same nose poke protocol to initiate water delivery and ensure that the rodent is looking at the stimuli and making a decision. The difference is that there are two water spouts, one underneath the center of each screen that are in a nose poke with a beam break. Once the rodent chooses, the beam break will record and the choice and signal for water delivery.

Design 3 has both screens on the far wall. There is still an initial nose poke for the initiation of water delivery and to ensure that the rodent sees the stimuli from the same position each time. The water delivery is the same as Design 2 where the water spouts and nose pokes are underneath each screen.

Design 4 includes the same screen setup as Design 3. In this design, there is only one nose poke with both water spouts, one for the right screen, one for the left. The beam break is still in the nose poke and will initiate water going into the spouts. Water will be delivered to the rodent and the choice between the stimuli will be recorded with a lick sensor (1) that will release the water in the spout. In the previous designs, the water spout runs down the wall and through a hole. In this design, the spout will come from the ceiling and connect to the nose poke.

Design 5 includes a similar screen setup as Designs 3 and 4, but instead of the screens being within the part of the cage that the rodent can move around in, it will be in a separate area along with the speaker. The wall that separates the cage from the screens will be opaque so that the only place the rodent can see the screens from is the nose poke hole, which will have a beam break sensor and both water spouts. This nose poke hole will function in the same way that the nose poke in Design 4 works with the lick sensors. The area containing the screens and speaker will be soundproofed.

The food dispenser is in the bottom left corner in every design and the speaker is to the left of the left screen in every design besides Design 5.

There are four alternatives to select from for the cage's feeding device: the Lafayette Instruments 20 mg Modular Pellet Dispenser (2), the Campden Instruments 45 mg Pellet Dispenser with Optional Stand (3), the Med Associates 20 mg Modular Pellet Dispenser (4), and the Cydnline Automatic Pet Feeder (5). The Lafayette dispenser attaches to a vertical panel and a short length of tubing for connects the body of the dispenser to the feeding area. A cylindrical container holds the dispenser’s pellet supply, and rotation of the outer wall of the cylinder allows dispersion of a set amount of food. Due to size constraints, this apparatus only reliably distributes 20mg pellets. The Campden Instruments 45 mg Pellet Dispenser with Optional Stand functions similarly to the Lafayette dispenser, with a cylindrical pellet container that rotates upon a voltage cue for food distribution. However, this dispenser does not require tubing and dispenses a wider range of pellet sizes - up to 45 mg. An optional stand is included with this Campden dispenser. The Med Associates 20 mg Modular Pellet Dispenser is calibrated for 20 mg pellets, with a capacity to hold ~7,000 for eventual distribution. The dispenser operates with a quiet 28 V DC stepper motor. For audio feedback with food dispensing, the Med Associates apparatus can be integrated with the “clicker module” ENV-135M. The Cydnlive Automatic Pet Feeder is modeled after a traditional hamster feeder, with a taller plastic cylindrical food container attached to a plastic dish. It is not programmable, but could distribute rodent pellets.

There are two identified alternatives for regulating water delivery in the RAT system: a Raspberry Pi-controlled valve and water reservoir setup (6), and the Jebao Programmable Auto Dosing Pump (7). In the first potential setup, a Mg996r motor - programmable by Raspberry Pi - rotates a valve connected to a water reservoir with the breaking of an infrared beam. The size of the water reservoir is customizable, as this option requires assembly of the setup from its components. The Jebao Programmable Auto Dosing pump (DP-2) is a pre-assembled option for regulating water delivery. The DP-2 has a pair of dosing heads, each programmable to dispense a precise amount of liquid up to 24 times a day. This pump includes its own battery power supply and requires calibration with a 100 ml graduated cylinder. Both of the discussed water delivery options for the RAT system require integration with the infrared beam break sensing systems of the proposed cage layout design.

When considering the optimal screen to display visual stimulus, there are three options. The first option is the Pi Powered Times Square Traveler Pack which is a 32x32 pixel LED screen controlled by a Raspberry Pi (8). The screen displays individual pixels but is also fairly large with a display of 15cm x 15cm. The second option is the PiTFT 2.8" TFT 320x240 Capacitive Touchscreen which is significantly smaller with only a 7.12 cm display but also provides touchscreen capabilities through capacitive sensors (9). The final option is the Standard LCD 16x2 White on Blue which is the simplest of the screens with a 2x16 character display that does not have touch screen capabilities (10). All of the screens listed are compatible with Raspberry Pi.

There are two key configurations for light and sound that both use the same products and a third configuration that uses an alternative light source. For the first two configurations, lights will be controlled by an Analog RGBW LED Strip (11) and sound will be controlled by the Stereo Enclosed Speaker Set (12). The first configuration with these products will have the speakers placed within the enclosed screen space proposed in Design 5 of Table 1 and the strip of LED lights lining that enclosed space. Alternatively, the same products could be used but with one speaker and a strip of LED lights inside the screen space, and another speaker and light strip outside in their living space. For both of these configurations, they will emit an automated sound and color light everytime that the rodent begins testing. The third configuration will use the same Stereo Enclosed Speaker Set but instead will use the Adafruit NeoPixel Digital RGM LED Strip with both stimulus in the screen space and living space (13). The new lights will line both the inside of the screen region and the living space within the cage. These lights will signal the start of testing for the rodent, but also can be controlled pixel by pixel allowing for the light source in the cage to shift throughout the day, similar to the sun going across the sky.

There are three alternatives when it comes to rodent interaction. The first option is to have no interaction at all between the rodents and have single cages. The second option is to have a shared wall with an LCD screen that can change between opaque and clear depending on the need at the time. The third option is a an opaque piece of glass. Both options two and three include having a small strip of laser cut class with holes underneath the rest of the wall so that the rodents can smell each other.

There are two alternatives for temperature and humidity monitoring. The first is the DHT11 arduino temperature and humidity sensor. This sensor, mounted on the cage's ceiling, will constantly monitor the temperature and humidity and will be programmed to send emails and text messages to the lab group if the temperature or humidity goes out of range. The other option is the DHT22 arduino temperature and humidity sensor. This will be programmed in the same way as the DHT11. The acceptable range for the Hengen lab is as follows: humidity is 30-70% and temperature is 20.5-26 °C. The difference between these sensors is as follows. The DHT11 is low cost, good for 20-80% humidity readings with 5% accuracy, good for 0-50 °C with ± 2 °C accuracy, can sample once every second (maximum), and has a body size of 15.5mm x 12mm x 5.5mm (14). The DHT22 sensor is slightly more expensive than the DHT11, good for 0-100% humidity readings with 2-5% accuracy, Good for -40 to 80°C temperature readings ±0.5°C accuracy, can sample once every 2 seconds (maximum), and has a body size of 27mm x 59mm x 13.5 mm (15).

ANALYSIS PERFORMED TO CHOOSE SOLUTION

A. Overall Design

Design 1 from Table 1 is beneficial because the screens can take up more space because they are on two different walls. Additionally, there is only one water spout so it minimizes the confusion the rodent may have by eliminating a variable: the rodent will always know that water will be coming from that nozzle. The drawbacks of this design are that there are three steps the rodent has to take before it gets water: first it has to look at the stimuli in the nose poke, next it has to make a decision by touching the capacitive sensor, and last it has to move to the spout to get the water. There are too many steps where the rodent could get confused or forget the choice it made causing it to not associate one task with another. The client is worried this method would take too long for the rodent to learn and the testing timeline would be significantly lengthened. The Pugh chart of Table 2 indicates that the components in Design 1 are not oriented ideally, as they generate an unnecessary number of tasks for the rodent to perform in training. This layout idea would be fairly easy to assemble, due to the simplicity of the water distribution, minimal number of beam break sensors and nose-pokes, and the known ease of assembly of capacitive sensors. However, the orientation of the screens at the intersection of walls means a rodent will perceive a different gradient on the near side of each screen compared with the far, thus undermining the validity of the forced-choice visual discrimination task. Design 1 additionally does not have in-cage soundproofing capacity, and was ranked fifth out of the available options in Table 2.

Design 2 from Table 1 has the same benefit of Design 1 with regards to screen size. It also has the benefit of having one fewer step than Design 1 between the rodent seeing the stimuli and getting water. The drawbacks of this design are similar to Design 1, specifically with the issue of perpendicular screens. Additionally, even though it has one fewer steps between stimulus and water delivery, the client would rather minimize the number of steps the rodent has to take to get water. Table 2 categorized Design 2 as neutral regarding the majority of evaluation criteria. This design improves upon Design 1 by coupling the rat's "decision indication" and water retrieval; instead of indicating its choice of screen by touching a capacitive sensor and then moving to the water source, in Design 2 the rodent can merely break the beam of the water source associated with the screen it chooses. Table 2 shows Design 2 ranked 4th.

Design 3 from Table 1 is beneficial because the screens are on the same plane so the gradients will be less distorted from the nose poke area. Additionally, the rodent has to go to the nose poke and then can go straight to the reward water - only two steps between the rodent seeing the stimulus and going to get water; fewer than Design 1. The drawbacks of this design are similar to Designs 1 and 2 in that the client wants to minimize steps between stimulus and water delivery. Table 2 shows the screen orientation comprises the difference between Design 2 and 3 - in Design 3, the linear mounting of the screens on a single wall help to ensure the rodent perceives the intended gradient while in the central nose-poke. However, the distance from this central-nose poke to the screens still allows for some variation in the rodents line of sight with the potential to influence gradient perception. The Pugh chart of Table 2 indicates the third place ranking of Design 3 out of the proposed options.

Design 4 from Table 1 is beneficial because the screens are on the same plane, and this has the minimum number of steps between stimulus and water delivery. The drawbacks to this design is that the tubing for the water spout would have to come from above and the client is concerned that the rats will tamper with the system. These changes from Designs 1-3 elevate Design 4’s proximity of components and rodent task number criteria evaluations in the Cage Layout Pugh Chart. The coupling of the two water screens, two water spouts, and central nose-poke eliminate the need for the rat to travel from where it views the screens to a different location to drink water. The ease of assembly of Design 4 is similarly improved in the Pugh chart, as this design needs two fewer nose-poke and infrared sensor complexes. Overall, Table 2 ranks Design 4 as the second most desirable cage layout.

Design 5 is beneficial because it restricts the rodent to only being able to see the stimulus from one place. Additionally, like Design 4, it has the fewest steps between stimulus and water delivery. This design minimizes the noise leakage from the speaker into other areas of the cage. This also fixes the issue from Design 4 because the tubing for the water spouts come down the wall that separates the general cage area from the screens. The drawback is that this region will decrease the amount of livable space for the rodent. Table 2 indicates the close orientation of components in Design 5, as the screens and water distribution system within the soundproof area are positioned immediately by the nose poke hole. This position allows for simultaneous screen viewing, decision indication, and water collection by the rodent, minimizing the rodent task number. Although Design 5 has a baseline ease of assembly - despite its relatively low number of components, it requires construction of an additional wall - the un-angled screen view and soundproofing potential elevate Design 5 above all other options. The RAT system's layout will abide by Design 5.

Cage Layout Pugh Chart

			Option 1	Option 2	Option 3	Option 4	Option 5
Evaluation Criteria	Weight	Baseline	Design 1	Design 2	Design 3	Design 4	Design 5
Proximity of Components	1	0	-	0	0	+	+
Minimal Rodent Task Number	2	0	-	0	0	+	+
Ease of Assembly	1	0	+	0	0	0	0
Un-angled Screen View	2	0	-	-	0	+	+
Soundproofing Potential	2	0	0	0	0	0	+
Total			-4	-2	0	5	7
Rank			5	4	3	2	1

Table 2. Pugh chart analyzing five different cage layout designs. The "+" and "-" signs correspond to "+1" and "-1" respectively in the calculation of the weighted totals for each option.

B. Feeding Device

The Pugh chart of Table 3 considers the four proposed options for the RAT system's feeding device. Of the four, the Campden Instruments 45mg Pellet Dispenser with Optional Stand (Pugh chart Option 2) ranked highest. This pellet delivery apparatus is entirely automated, requiring no manual manipulation aside from the initial filling of the chamber with pellet. Two of the other options include equivalent electronic automation, while Option 4 harnesses gravity to distribute pellets and potentially enables over-eating. Option 2's ease of programming was indicated as neutral in Table 2, as the RAT system does not require super fine control of feeding, and the original settings of the device should be sufficient. Parallelling their similar automated function, Option 1, 2, and 3 received equivalent ratings for "Ease of Programming." The Cydnlive

Automatic Pellet dispenser cannot be programmed. Assembling the Campden feeder would be easier than Option 1, which requires manually mounting to a wall and incorporation of tubing. The cost of the Option 2 varies depending on demand; however, the eBay lists the price of this feeder at $170. The cost of this feeding system is worth its automated features and ability to handle the pellet sizes used in the Hengen lab, provided Dr. Hengen is willing to fund the purchase of this device.

Feeding Device Pugh Chart

			Option 1	Option 2	Option 3	Option 4
Evaluation Criteria	Weight	Baseline	Lafayette Instruments 20mg Modular Pellet Dispenser	Campden Instruments 45mg Pellet Dispenser with Optional Stand	Med Associates 20mg Modular Pellet Dispenser	Cydnlive Automatic Pellet Dispenser
Automated Delivery	2	0	+	+	+	-
Ease of Programming	1	0	0	0	0	-
Ease of Assembly	2	0	-	+	0	+
Cost	2	0	0	-	0	+
Optimal Size	1	0	-	+	-	+
Weighted Total			-1	3	1	2
Rank			4	1	3	2

Table 3. Pugh Chart Analyzing Feeding Devices. The “+” and “-” signs correspond to “+1” and “-1” respectively in the calculation of the weighted totals for each option.

C. Watering Device

The RAT system will incorporate Option 1 of Table 4. Option 1 - an Arduino-controlled valve and water reservoir- is ranked higher than the Jebao pump, mostly due to its lower cost. The Jebao pump incorporates unnecessary features for fine-tuning the timing and amounts of water delivery. The RAT system’s design, however, necessitates a watering device that can be programmed to release water in response to an infrared beam break. The capability of Option 1 to integrate with these requirements is reflected in its positive “Ease of Programming” qualification in the Pugh chart of Table 4.

Water Device Pugh Chart

			Option 1	Option 2
Evaluation Criteria	Weight	Baseline	Raspberry Pi-Controlled valve and Water Reservoir	Jebao Programmable Auto Dosing Pump
Automated Delivery	2	0	+	+
Ease of Programming	2	0	+	0
Ease of Assembly	1	0	0	+
Cost	2	0	+	-
Optimal Size	1	0	+	+
Weighted Total			7	2
Rank			1	2

Table 4. Pugh chart analyzing two different water devices. The "+" and "-" signs correspond to "+1" and "-1" respectively in the calculation of the weighted totals for each option.

D. Screens

As indicated by Table 5, the optimal choice of screen is the PiTFT 2.8" TFT 320x240 Capacitive Touchscreen. Although the Pi Powered Times Square Traveler Pack allows for a clear display across the range of pixels, this large display is also its downfall. The display is so large that it would occupy almost the entire wall of the cage, preventing two screens from being able to be compared side-by-side. Another problem with this product is the high price tag. Since cheaper alternatives are available for similar or higher quality, the high price can not be justified within the context of this system. The Standard LCD 16x2 White on Blue is conversely extremely cheap but the display is oversimplified. While the display is sufficient for words, it will not allow for the proper presentation of slightly varied gradients of visual stimuli. Lastly, the PiTFT 2.8" TFT 320x240 Capacitive Touchscreen proves to be the most successful option due primarily to its ideal size and low cost. The benefit of the Capacitive Touchscreen is that the display is extremely clear while being only 7.12cm, allowing for two screens to be displayed side-by-side for each comparison by the rodent. Although this design is beneficial, it is not desired for the screen to be a touchscreen primarily because the rat should not be able to alter the screen or manipulate it in any way. Since the final design of the cage will reflect Design 5 in Table 1, the screens will be physically separated from the rodents in a distinct section of the cage therefore the rodent will not be able to touch the capacitive screen or interfere with its display. The Screen Pugh chart rates each of these screen options on the relevant design criteria, ultimately ranking Option 3 - PiTFT 2.8” TFT 320x240 - first out of the set.

Screen Pugh Chart

			Option 1	Option 2	Option 3
Evaluation Criteria	Weight	Baseline	Pi Powered Times Square Traveler Tack	Standard LCD 16x2 White on Blue	PiTFT 2.8" TFT 320x240 + Capacitive Touchscreen for Raspberry Pi
Ease of Programming	2	0	+	+	+
Optimal size	1	0	−	0	+
Cost	1	0	−	+	+
Gradient Display Capability	2	0	−	−	+
Weighted Total			-2	1	6
Rank			3	2	1

Table 5. Pugh chart analyzing four different screens. The “+” and “-” signs correspond to “+1” and “-1” respectively in the calculation of the weighted totals for each option.

G. Light and Sound

Upon consideration of the three alternatives for the light and sound configuration, option 2 was determined to be the best. The first configuration with the light and sound only placed in the screen space is a good option because it allows the additional stimulus to be exclusively associated exclusively with testing time and prevents neighboring rats from being exposed to the same sounds. Therefore a noise and light can be emitted when testing begins and a different noise and light can be emitted for the correct decision. By having these stimuli isolated from any neighboring rodents, it is predicted that the rodents will learn to make the correct choice quicker. One weakness of this design is that the rodent cannot be exposed to the light and sound before they begin testing therefore the stimulus cannot be used as a trigger to tell the rodent to begin the test. This weakness is addressed by the second configuration which allows the speakers and light to also be in the rodent’s living space. One tremendous benefit of having the light and sound in the living space is that eventually, their signal can tell the rodent that testing is about to initiate and this will trigger the rodent placing its own head in the nose poke. A weakness of this design is that by having the light and sound out in the open living space, it is likely that neighboring rodents will be exposed to these signals and may confuse them with their own. To prevent this problem, each cage can be programmed to have a distinct light and sound associated with it so that the neighboring rodents recognize that it is not their stimulus. With this modification, the second configuration proves to be superior. The third configuration uses the neopixel light strip, allowing for the light to not only control stimulus associated with testing, but also to control the light in the cage to emulate the sun going across the sky as the central light source passes across the cage throughout the day. The benefit is that the client would then be able to influence the circadian rhythm of the rodent and potentially perform experiments when this natural rhythm is altered. The weakness of this design is that having the representation of the sun go across the small cage throughout the day would be an accurate representation of nature. Instead it would be beneficial for the artificial light source to travel across the room throughout the day which is outside the scope of this project and will instead be actively pursued by the client.

E. Temperature and Humidity Monitoring

The benefits of the DHT11 is its slightly faster sampling rate, slightly lower cost, and smaller size than the DHT22. However, the DHT22 has a larger range of temperatures and humidities and is more accurate than the DHT11. Additionally, the Hengen lab has a number of DHT22s that can be used in this project, so it makes the most sense to go with the DHT22. Table 6 compares the DHT11 and DHT22, ultimately ranking the DHT22 higher based on the chosen weighted criteria.

The DHT is rated positively for all criteria, with exception of its size, which is slightly larger than the DHT11. However, mounting this sensor at the top of the cage will render this very slight increase in size inconsequential.

Temperature and Humidity Sensor Pugh Chart

			Option 1	Option 2
Evaluation Criteria	Weight	Baseline	DHT11 Arduino Sensor	DHT22 Arduino Sensor
Accuracy	2	0	-	+
Reading Range	2	0	0	+
Cost	1	0	+	+
Optimal Size	1	0	+	0
Sampling Rate	1	0	-	+
Weighted Total			-1	6
Rank			2	1

Table 6. Pugh chart analyzing two different temperature and humidity sensors. The “+” and “-” signs correspond to “+1” and “-1” respectively in the calculation of the weighted totals for each option.

F. Rodent Interaction

The benefits of rodent interaction during the study were discussed both with the client and with others in the field doing similar studies. The consensus was that rodent interaction is critical for creating the most natural environment for the rodent during an unnatural study. Thus, the design will include a shared wall for rodent interaction. The benefits of an LCD wall that can change between opaque and clear is that when the rodents are not training, they can both see and hear each other. The benefits of the opaque wall is that it is less expensive and simple to implement.

CHOSEN SOLUTION

After careful consideration of all the alternatives, the following overall design was determined to be optimal. The layout of the cage will follow Design 5. The food dispenser will be the Campden Instruments 45mg Pellet Dispenser with Optional Stand. The water dispenser will be an Arduino-controlled valve and water reservoir. The screens will be two PiTFT 2.8” TFT 320x240 Capacitive Touchscreens. The light and sound configuration will be configuration 2. The temperature/humidity sensor will be the DHT22 sensor. There will be a shared opaque wall with holes at the bottom for the rodents to smell each other.

PROPOSED BUDGET

Category	Item	Cost per Item	Quantity	Total Cost
Food/Water	Water Spout	$32.50	2	$65.00
	Water Valve	$6.95	2	$13.90
	Food Dispenser - Campden Instruments 45mg Pellet Dispenser	$169.99	1	$169.00
Lick detector	Relay: EDR SPST DIP relay, 500 ohm coil (Jameco PN 106463).	$2.50	2	$5.00
	Relay socket: 14 pin DIP (Jameco PN 526192).	$0.05	2	$0.10
	R1 and R2: 10M ½ watt resistors (Jameco PN 662477).	$0.04	100	$4.00
	R3: 47K ½ watt resistor (Jameco PN 661917).	$0.04	100	$4.00
	PN 178511).	$0.20	2	$0.40
	Multipurpose PC Board 417 Holes	$2	2	$4.00
Beam Break	Sensors	$1.95	1	$1.95
Screen	PiTFT 2.8” TFT 320x240 Capacitive Touchscreen	$5.08	2	$10.16
Light and Sound	Analog RGBW LED Strip	$19.95	2	$39.90
	Stereo Enclosed Speaker Set	$7.50	2	$15.00
Temperature and Humidity	Heat lamp	$36.75	1	$36.75
	Temperature/Humidity sensor	$5	1	$5.00
Rodent Interaction	Wall	$50	1	$50.00
System Control	Arduino Uno	$25	1	$25.00
	Raspberry Pi	$40	1	$40.00
			Total Cost	$489.16

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