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Assessing Visual Perception Using Behaviour Conditioning in the Rat Model

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Assessing Visual Perception Using Behavior Conditioning in the Rat Model

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ABSTRACT

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Assessing Visual Perception Using Behavior Conditioning in the Rat Model

Thesis Advisor: Dr. Jameel Ahmed

Neural prosthetics aim to restore function to sensory deficits. In the same sense that cochlear implants can restore auditory function, visual neural prosthetics aim to restore visual function. A strain of rats with retinal degeneration are subjects of great interest when exploring the effect of a visual neural prosthetics on visual perception. In this study we explore the rat response to a visual stimulus in normal vision rats through behavior conditioning in the development of a training protocol that will be used to assess visual perception in retinal degenerative rats. We found that autoshaping was a successful method in training rats to form an association between lever presses and food delivery. We also found that light discrimination under a two-lever, one-wall paradigm resulted in strong subject response to introducing light dependent food enforcer delivery. Further exploration of visual perception in rats under this paradigm was unable to be performed.

ACKNOWLEDGEMENTS

I would like to thank Jameel Ahmed as my research mentor. Thank you to Shannon Tieken for her work as animal care manager. Thank you to Tom Rogge for construction of the testing chamber. Thank you to Ella Ingram for statistical analyses assistance and the rest of ABBE faculty for support and guidance during junior proposal and senior thesis development. Lastly, thank you to the Joseph B. and Reba A. Weaver Undergraduate Research Grant, IRC, and Lilly Guidant Applied Life Sciences for funding.

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INTRODUCTION

Neural prosthetics have demonstrated success in restoring function to those with sensory deficits. For instance, cochlear implants are able to restore auditory function through stimulation of the auditory nervous system when damage occurs to the hair cells in the cochlea [1]. In the same sense, visual neural prosthetics have the goal of restoring function to visual deficits when eyes no longer function [2].

There exists a strain of rats whose visual function is lost as their retinal thickness degrades over time. Degeneration of the retina is a result of a mouse opsin gene containing a termination codon at residue 334. This results in an opsin protein lacking the last 15 amino acid residues at the C-terminal, which serve as sites for phosphorylation [3]. These rats are of high interest as subjects for visual neural prosthetic development. In developing this study we asked the question: can rats with retinal degeneration perceive a light stimulus using a prosthetic after vision is lost? Unfortunately, we currently lack the tools to answer this question. However, in order to answer this question we must first determine the ability of normal vision rats to perceive a light stimulus.

When assessing perception of stimulus in human subjects, subjects are able to respond with direct communication. However, this same assessment cannot be applied to animal subjects. Subject perception can be assessed using behavior conditioning. Behavior conditioning functions on the idea the subjects react to a stimulus in the form of a conditioned response. In this study we focus on two forms of behavior conditioning: autoshaping and operant conditioning. Autoshaping focuses on forming an association between an unconditioned stimulus with a conditioned stimulus. An unconditioned stimulus is one that elicits a natural response from a subject known as the unconditioned response. For example, the unconditioned stimulus of food

results in the unconditioned response to eat. A conditioned stimulus is an initially neutral stimulus that later becomes associated with the occurrence of the unconditioned stimulus. For example, light as a stimulus does not initially elicit a response from a subject, but after an association is formed between light and the unconditioned stimulus of food, subjects are observed to display food seeking behavior in response to the light stimulus. The pairing of the two stimuli, however, occurs regardless of the response from the subject and results in the desired subject behavior to be self-shaped [4]. Operant conditioning focuses on encouraging behavior through reinforcement and discouraging behavior through punishments [5]. The current study only focuses on shaping subject behavior through positive and negative reinforcement. In positive reinforcement, a food enforcer is delivered when the correct behavior is observed. Under negative reinforcement, the food enforcer is withheld when the incorrect behavior is performed by the subject.

Rats are a standard species for investigating behavioral responses. Extensive research has been performed in vision-mediated behavioral tasks using rat subjects. Peterson et al were able to direct motor responses through autoshaping through use of a brain stimulus reinforcer to train rats to respond to a visual stimulus with the pressing of a lever [6]. In addition, Atnip trained rats, under autoshaping and operant conditioning, to press a lever using a food reinforcer [7]. Using a combination of methods described in these studies, the present study aims to develop a protocol using behavior conditioning to assess perception of a light stimulus in rats. In this study, subjects are conditioned to respond to a light stimulus by pressing a lever in order to receive a food enforcer.

METHODS

Subjects

Two Sprague Dawley rats, JA-28 and JA-33, were examined for their response to visual stimulus to assess feasibility of use as test subjects in studies that measure visual perception. Subjects did not demonstrate the necessary visual acuity for testing parameters and were not used for any further testing. Experiments described in this study were performed on two male Blue Spruce Long Evans rats, JA-35 and JA-36 (~260g in mass, Harlan Laboratories). Prior to each training session, subjects were placed on a 12-hour food-deprivation schedule. Subjects were placed on a swimming exercise schedule at the beginning of the Two-Wall Forced Choice training phase (Appendix A).

Test Chamber

The rats were trained in a 12 x 9 x 10 in (L x W x H) testing chamber with black, matte, opaque walls and clear plexiglass hinged lid (Figure 1A). During behavior training, wall(s) were situated with a lever switch (Jameco VM-05K-03D0), a port through which an LED (Jameco MCDL-L20-350HPCWS) was shown, a translucent light diffuser (8mm diameter), and a feeding port where the food reward was delivered into a feeding cup (Petco plastic bird cage cup). A schematic for the arrangement of the chamber fixtures is depicted in Figure 1B.

Training Methods

Prior to each trial of testing, the chamber was disinfected with Great Value brand spray disinfectant and hinged lid left open for five minutes to aerate prior to insertion of the subject. Upon insertion, subjects were allowed thirty to sixty seconds of adjustment time prior to beginning the training trial. The subjects underwent several phases of training in order to explore

the visual perception. A flowchart depicting testing phases and the relevant phase stimuli is depicted in Figure 2. Early training methods aimed to train rats to eat in the testing chamber. Subjects underwent training for three ten- minute trials and received food delivery through a food port into a feeding cup.

Baseline Testing

Baseline information was then gathered for the subjects' natural lever press behavior during five twenty-minute trials. Subjects were placed in the test apparatus with the lever switch protruding from wall A (Figure 1A). The feeding cup was not in place and the food port was covered to prevent early association of lever pressing with food reward.

Autoshaping

The next stage of training utilized the behavior conditioning method of autoshaping to form the association between lever pressing and food delivery in seven twenty-minute trials. The tenet of autoshaping is that the development of the desired subject behavior is self-shaped. Thus, food delivery is not dependent on the subject pressing the lever. Food was delivered on a twenty second schedule and subjects were observed for the total number of lever presses in the twenty-minute trial. The beginning of each trial was signaled by the insertion of the feeding cup.

Constant Light

Rats were trained in several variations of the stimulus-response task. During the first phase, I observed the effect of a constant light stimulus on subject response. The testing chamber was fit with a response lever, a feeding cup, and a mounted LED where light shone through an uncovered port. The arrangement of the chamber materials in depicted in the Wall A arrangement of Figure 1B. A constant stimulus was activated and controlled through LabScribe

software (setup described in Data Recording section). In the 20-minute trial, food delivery occurred every twenty seconds and rats were observed for the number of lever presses. Four trials were performed.

Autoshaping with Light

In the next phase of stimulus dependent testing I employed autoshaping methods and varied the rate of stimulus firing. In the eight 20-minute trials, a pulse light of amplitude 5V fired every 6.5 seconds with duration of 6.5 seconds, the maximum automated pulse stimulator value allowed by the LabScribe software. Food was delivered during light activation and was not dependent on whether subjects pressed the lever. Subjects were observed for the frequency of light-on pressing.

Operant Conditioning

Only positive and negative reinforcement were used during this phase due to ACUP (Appendix A) restrictions. Food delivery occurred when the rat pressed the lever when the light was activated and food delivery was withheld when the lever was pressed outside of light activation. The testing chamber is modeled in the Wall A setup of Figure 1B with exception of the removal of the feeding cup. The beginning of trials from this point forward were signified with the firing the light stimulus. In the five twenty-minute trials, a pulse light stimulus was manually driven to fire every 20 seconds with duration of 4.5 seconds.

Two Wall Forced Choice

Chamber testing feature arrangement is depicted in the Wall A and C portion of Figure 1B. Subjects have the natural tendency to respond to the setup of Wall A, thus preliminary trials aimed for rats to press each lever with equal proportions to reduce this preference. Three 20-

minute baseline training trials were performed independent of light stimulus. During these, rats were rewarded with food when they correctly alternated approaching and pressing levers on Walls A and C. Light stimulus was implemented in the subsequent two trials. The stimulus was manually driven to fire every 15 seconds on alternating sides with duration of 4.5 seconds. Rats were rewarded when lever presses occurred during the firing of the light stimulus.

One Wall Forced Choice

The testing chamber was modified as depicted in the Wall B portion of Figure 1B. A pulse stimulus of amplitude 3V with 6.5 second duration was manually driven to fire every 30 seconds, alternating between levers A and B. In the three twenty-minute trials, subjects were rewarded when the lever presses matched the stimulus activation time and location.

DATA RECORDING METHODS

During the first two trials of baseline lever data collection, I recorded the lever press data by hand using tallies to signify the number of lever presses per minute. Time was recorded with a timepiece (VWR). Recordings by hand were performed because an automated system for data recording had not yet been developed. Subsequent trials utilized iWorx equipment (IWX 214) and LabScribe (v.2) software for data collection. A Matlab code was generated to analyze the LabScribe data (Appendix C).

Levers and LEDs are connected to a breadboard in two separate circuits (Figure 1B) and are powered by a dual output DC power supply (Agilent E3620A). LEDs and levers were connected to the stimulator channels and Channel 1 of the iWorx module, respectively. Trials in which more than one lever was used, a second iWorx module and computer were used for data recordings. Light activation and lever press data are recorded as changes in potential over time.

Raw data from each trial recording were exported into a text file for every 50 data points, resulting in 2400 data points for code analysis per trial. These data were imported into a written Matlab code "Analysis.m". The code imports the raw data values and analyzes for changes in potential and exports values of lever pressing frequency and counts the number of light firings. Calculations are exported into an Excel file (Appendix B) and these data are analyzed in Minitab.

STATISTICAL METHODS

Data were analyzed using Minitab. All tests were performed at 95% confidence. Analyses for comparing the effect of various factors on the response of the subject utilized a General Linear Model (GLM) test tailored to a repeated measures analysis. A repeated measures analysis was used because these data were collected from the same subjects over several time points. One sample t-tests were used to determine whether subjects could discriminate and develop a preference for light. The ratio of the number of lever presses per second when the light was on versus the number of lever presses per second when the light was off was calculated in order to analyze the subject response to light. A comparison ratio value of 1 was used and signifies that the frequency of lever pressing is equal for when the light is on and off. Ratio values greater than 1 suggest subjects show a preference for the presence of the light stimulus. Development of preference for light stimulus is signified by an increase in the ratio over subsequent trials. Missing ratio values that resulted from a zero frequency of off presses (Appendix B) were replaced with the rounded maximum ratio for that trial to generate a "true" ratio. Minitab output for statistical tests performed on data is located in Appendix D. Table 1 contains the GLM parameters and additional test parameters examine for all the statistical tests performed.

RESULTS

Baseline and Autoshaping

Both subjects demonstrated an increase in learning when trained using the autoshaping paradigm to press a lever. Learning is signified by an increase in the average number of lever presses when comparing the baseline and autoshaping no light data. In baseline trials subjects averaged 22 ± 3 (mean ± standard deviation) lever presses per trial and 135 ± 88 per trial during the autoshaping paradigm (Figures 3 and 4, respectively). Training phase had an effect on the total number of presses observed ($p<0.05$, $t=5.42$).

Constant Light

The presence of the constant light stimulus did not affect the average number of lever presses when the mean lever presses of constant light trials were compared with the last four trials of autoshaping trials ($p>0.05$). A figure of these result was not generated.

Autoshaping with Light

Using autoshaping coupled with light to introduce the light stimulus did not result in a greater preference for lever pressing when the light was on. Ratios over subsequent trials were not observed to increase over time ($p>0.05$). Subjects demonstrated different preferences for the light stimulus ($p<0.05$). Subject 36 demonstrated a greater preference for the presence with an average ratio of 2.12 while subject 35 demonstrated an average ratio of 1.58. Both subjects were able to distinguish the presence of the light stimulus. Average lever press frequency ratios for all trials under this testing phase are greater than 1.

Operant Conditioning

Subjects did not develop a preference for the light stimulus under the operant conditioning paradigm. Ratios for press frequency did not increase over subsequent trials ($p>0.05$). Under this testing phase, subjects did not demonstrate a preference for the presence of the light stimulus. The average ratio for all trials for subjects was not greater than 1 ($p<0.05$, $t_{35}=0.21$; $t_{36}=-3.77$).

Two Wall Forced Choice

Subjects did not develop a preference for the light in the two wall discrimination trials. Average ratios for all trials did not increase over subsequent trials ($p>0.05$). Subjects demonstrated a preference for the light stimulus ($p>0.05$). The effect of the lever location was assessed for an effect on the ratio of pressing frequency. Subjects did not demonstrate a preference for either lever ($p>0.05$). Combining lever data for these trials did not affect the ratios at any factor ($p>0.05$).

One Wall Forced Choice

The subjects demonstrated a strong response to the one wall discrimination task. Subjects demonstrated a preference for the light stimulus ($p<0.05$). Not enough trials under this paradigm were performed to conclude a significant increase in the ratio values of time ($p>0.05$). However, the combined ratio values under the one wall paradigm are observed to be greater than the combined ratio values under the two wall paradigm ($p<0.05$).

DISCUSSION AND CONCLUSION

The autoshaping paradigm of behavior conditioning worked as a mechanism for training rats to press a lever. This result is consistent with work on rats from Atnip, who was able to use a food enforcer to train rats in pressing a lever. This outcome can lead to strategies for assessing perception in organisms without relying on direct communication. These strategies can be applied to assessing the efficacy of neural prosthetics in restoring function to sensory deficits in animal subjects.

Autoshaping with the light stimulus results were expected to be more successful than observed. Work from Peterson et al were able to utilize autoshaping in rats to generate light stimulus dependence of lever pressing with brain stimulation reinforcement. This result may be attributed to the design in the training scheme. Rats were trained in several phases: first with lever pressing and food, and then the inclusion of the light stimulus. Because the light stimulus was not incorporated from the beginning of the testing scheme and subjects had already formed an association between the lever press and food delivery, no motivation was present to form the association with the light stimulus. This lack of motivation is a result of the autoshaping paradigm because the association of stimuli occurs independent of subject response.

Operant conditioning to introduce the light stimulus dependence was expected to be more successful. We hypothesize that the lack of development of the stimulus preference was a result of only using positive and negative reinforcement instead of exploring the punishment aspect of operant conditioning. These findings are consistent with the work of Warden et al in which punishments and reinforcements were explored for their effect on subject performance in visual

discrimination [11]. These researchers found that a combination of positive reinforcements and positive punishments is most effective in conditioning the desired behavior. Punishments and reinforcemnts in this study were not pursued due to restrictions in animal testing as outlined in ACUP approval. Future work of this study, therefore, may use positive punishment and positive reinforcement to shape subject behavior. In addition to the ineffective behavior training parameters for operant conditioning, subjects under this phase were observed to increase in their distracted behavior. Because of the testing parameters in this study were similar in regard to the setup of the testing chamber, distracted behavior may have resulted from habituation of the task. File et al observed that habituation occurs when there is a lack of novelty in the task. The opposite effect was observed in later phases when the testing parameters changed, suggesting habituation is a strong factor in affecting subject response. As a result, it may be more effective to change the testing parameters more frequently or utilize more than one set of test subjects for each phase of testing in future work in order to reduce habituation.

Training rats to respond to the light stimulus was most successful in the one-wall setup. Better performance in this setup may be attributed to the amount of light the subjects were able to observe and the better orientation of the subjects. During the two-wall setup, subjects were observed to have difficulty orienting to the correct location of the lever and light stimulus if focused on the other lever. It remains undetermined if subjects in the two-wall setup were responding to the reflection of the light stimulus on the opposite wall, which may have led to incorrect location pressing. Literature searches were not successful in determining rat sensitivity to a light stimulus; however sensitivity to reflection on the opposite wall can be examined experimentally. This would require photometer measurement of the light reflection and utilizing that measurement in observing the subject response to a single stimulus of that light intensity.

Better performance in the one-wall setup may also be attributed to another aspect of test chamber design. During construction of the chamber in the two-wall setup, black caulk was used to fill a larger accidental cut made in the wall. During training, subjects demonstrated highly distracted behavior and high interest in the black caulk. The distracted behavior during these test phases was quantified by a fifty-percent reduction in the total number of lever presses when compared to Operant Conditioning single lever trials. Further pursuit of this training phase was halted and a redesign of the test chamber occurred to place the two levers on a single wall.

This study aimed to develop a training protocol for assessing visual perception in rats. While I was not able to explore the development of the protocol any further to gauge learning of the light discrimination because of time constraints, I was able to determine the most effective methods for implementing light stimulus dependency in subject response. In 1981, Passe demonstrated success in using behavior conditioning to assess the limitations of the visual system in pigeons [9]. Upon optimization of the training protocol, these methods will be pursued to examine how rats with retinal degeneration perceive a light stimulus from a neural prosthetic device.

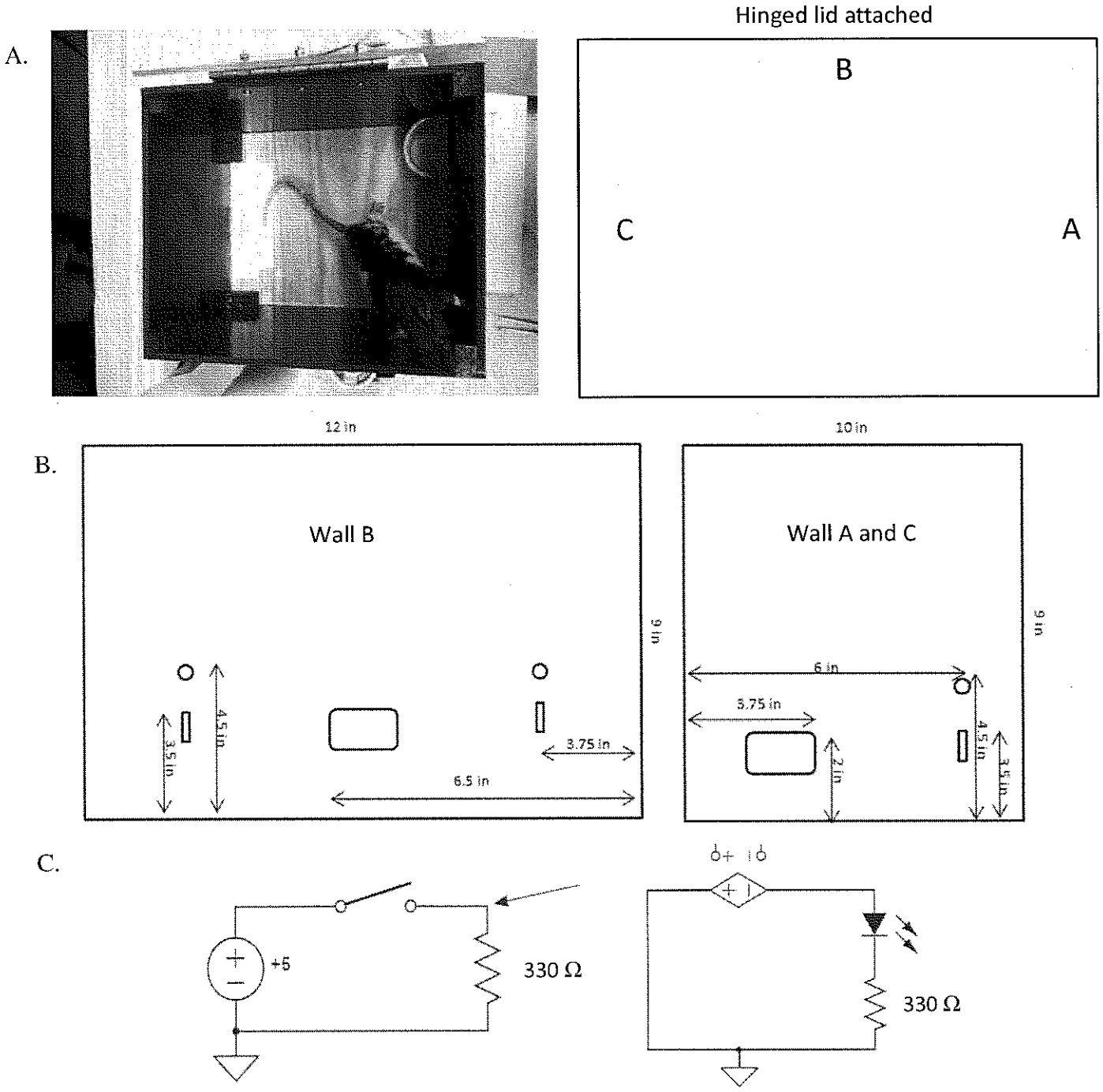

Figure 1. Testing Chamber Set-up. A. Aerial view photograph of testing apparatus (left) and equivalent schematic of wall arrangement (left). B. Schematic arrangement of testing chamber fixtures: light stimulus (circle), food port (curved edge quadrilateral), and lever arm insertion slot (thin quadrilateral). C. Circuit arrangement for the lever (left) and light stimulus (right). The lever circuit was powered by a D/C power supply at 5V. Pressing the lever completed the circuit and resulted in an observed change in potential in LabScribe software. Reading of the circuit occurred at the location of the arrow. The light stimulus circuit was driving by a variable voltage source in with the iWorx module and LabScribe software. Firing of the voltage source resulted in firing of the LED light stimulus.

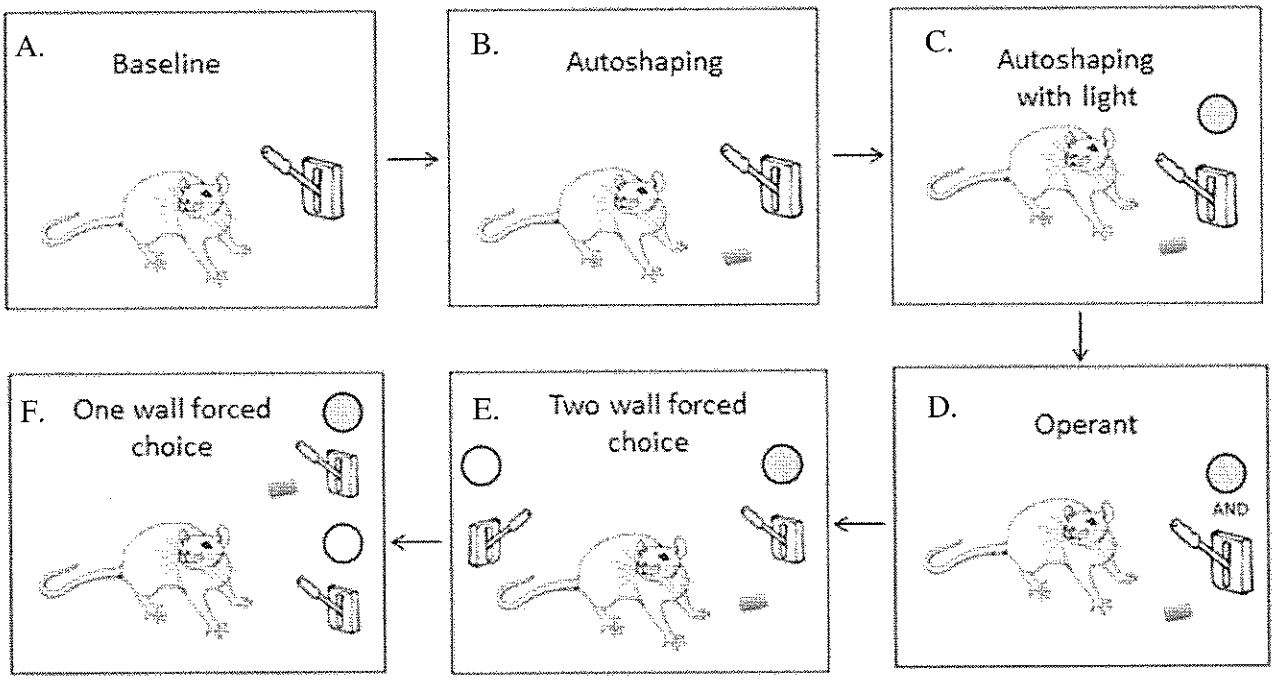

Figure 2. Flow Scheme of Behavior Training Procedures and Test Chamber Fixture Arrangement. A. Baseline trials contained a single lever fixed to Wall A of the test chamber. Subjects were observed for their natural response to the lever. B. Autoshaping trials aimed to form the association between lever press and food delivery. The testing chamber was fixed with a single lever and a port for food delivery. C. Autoshaping trials aimed to incorporate the association of the light stimulus with the delivery of food and subjects were to respond by pressing a lever. Lever press did not affect the delivery of the food and was dependent on the firing of the light stimulus. The test chamber contained a lever, a food port, and a light. D. Operant conditioning trials aimed to train subjects to be dependent on the firing of the light stimulus in order to receive the food enforcer. Delivery of the food enforcer required lever press to match the time the light stimulus fired. E. The two wall forced choice paradigm aimed to train rats to be able to discriminate the presence and location of the light stimulus. Delivery of the food enforcer required subjects to press the lever in the correct location and time the of the light stimulus firing. The test chamber was arranged so two levers were located on opposite walls (Walls A and C). F. One wall forced choice aimed to develop the light stimulus dependence. In order for the food enforcer to be delivered, the subjects had to correctly match the location and firing of the light stimulus. The test chamber was arranged so two levers were on a single wall, each with its own light stimulus above the lever. A food port was in the center of the two levers.

Condition	Subject [fixed] (levels)	Trial (levels)	Phase (levels)	Lever (levels)	Response	Additional Test Results
Baseline and Autoshaping	✓(2)	✓ (9)	✓(2)		Total Press	$\mu_{auto} = \mu_{baseline}$[1]
Constant Light and Autoshaping	✓(2)	✓(4)	✓(2)		Ratio	$\mu=1$[2]
Autoshaping with Light	✓(2)	✓(8)			Ratio	$\mu_{35} = \mu_{36}$[1] $\mu=1$[2]
Operant Conditioning	✓(2)	✓(5)			Ratio	$\mu=1$[2]
Two Wall Forced Choice	✓(2)	✓(2)		✓(2)	Ratio	$\mu=1$[2]
Two Wall Forced Choice (Combined Levers)	✓(2)	✓(2)			Ratio	$\mu=1$[2]
One Wall Forced Choice	✓(2)	✓(3)		✓(2)	Ratio	$\mu=1$[2]
One Wall Forced Choice (Combined Levers)	✓(2)	✓(2)			Ratio	$\mu=1$[2]

[1]Two-sample t-test (one-sided)
[2]One-sample t-test

Table 1. GLM Parameters for All Statistical Tests. GLM test compared the data for the conditions for the effect of possible factors Subject, Trial, Phase, and Lever on the condition response at 95% confidence. Subject as a factor tested variations in subject performance. Trial as a factor signified repeated measures and analyzed the change in subject response over time. In tests where Phase was a factor, multiple conditions were examined in the same test and effect of each condition was examined on the response. Lever was a factor in the One Wall and Two Wall Forced Choice paradigms and measured the effect of each lever on response. Each factor is broken down into levels. The number of lever for each factor is depicted in parentheses. Additional statistical tests performed were one-sample and two-sample (one sided) t-tests. The null hypotheses used in each of these tests included.

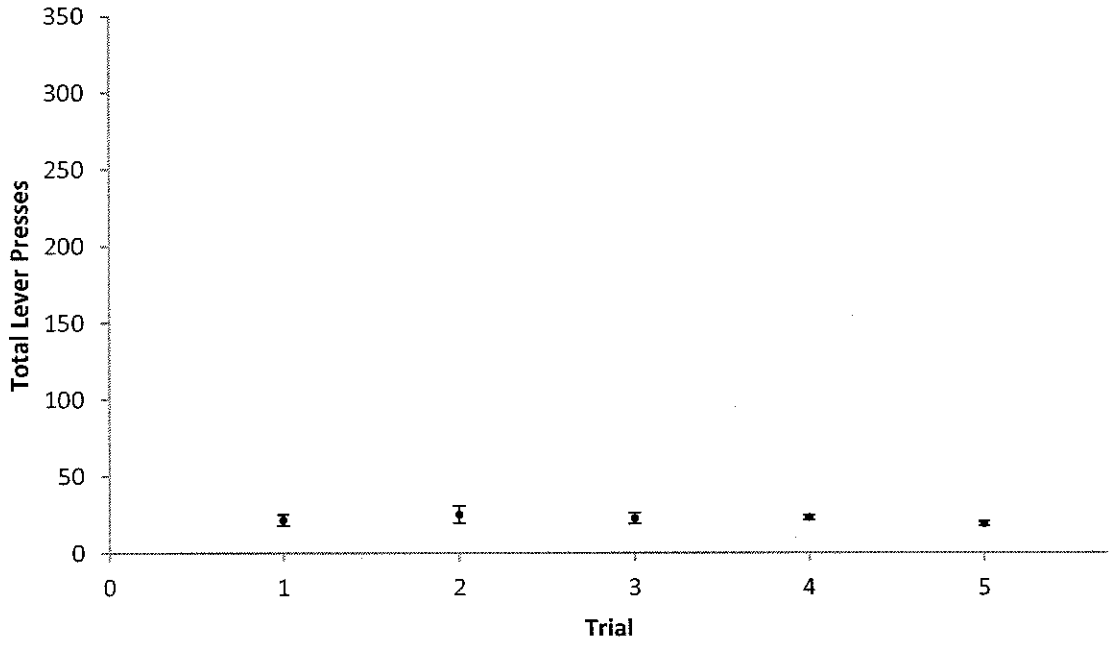

Figure 3. Baseline Testing Results. Subjects were observed for natural response to lever without reinforcement delivery. Average values of total number of lever presses for the two test subjects are plotted with closed circles with bars of standard deviation. Subjects averaged 22 ± 3 lever presses for each trial and did not demonstrate an increase in the number of lever presses over time.

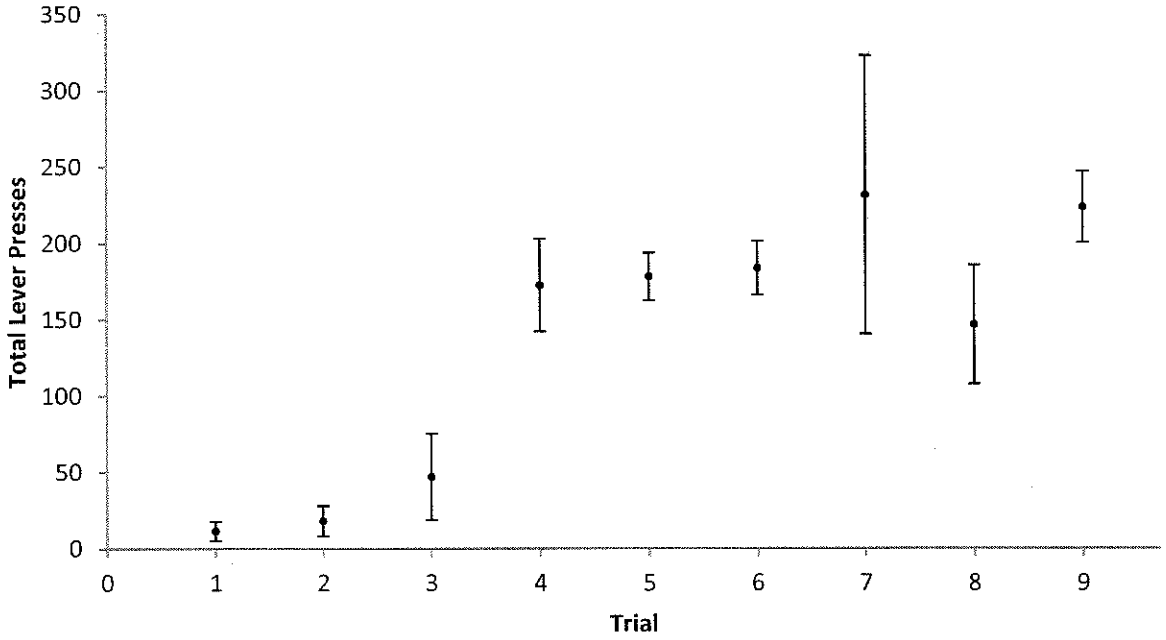

Figure 4. Autoshaping Results. Subjects were trained under the autoshaping paradigm to form the association between lever press and food delivery. Average values of lever presses per trial are plotted with closed circles and bars of standard deviation. Subjects averaged 135 ± 88 lever presses per trial. Subjects demonstrated learning of the task, signified by the increase in average lever presses subsequent trials.

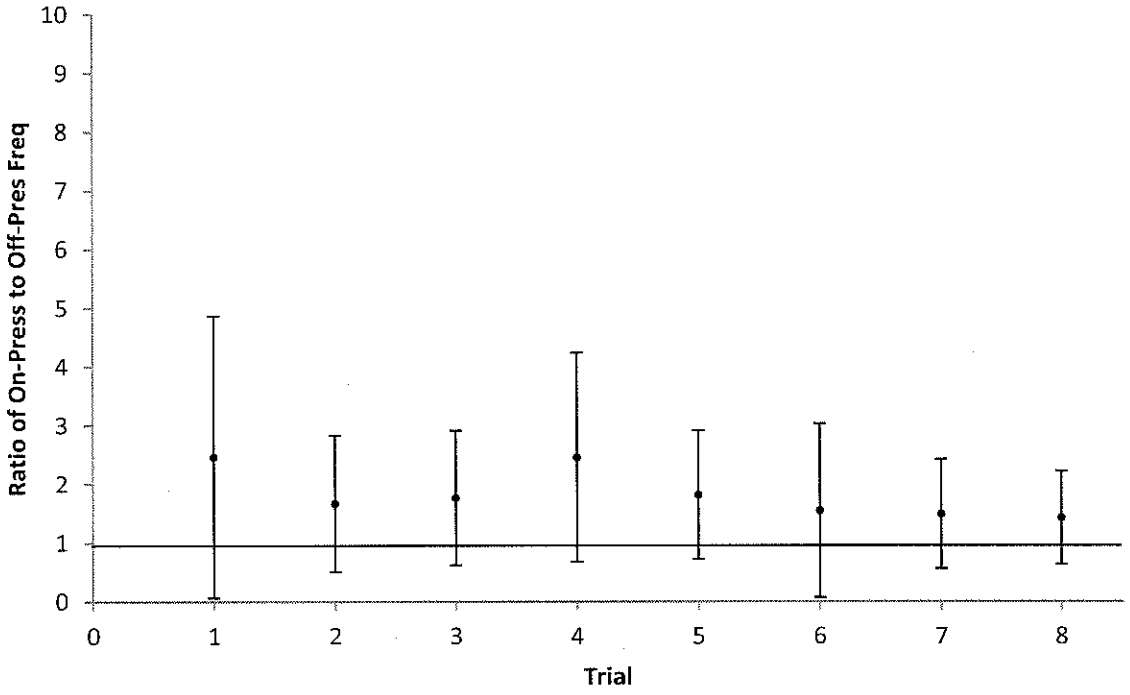

Figure 5. Autoshaping with Light Stimulus Results. Subjects were trained under autoshaping paradigm to form association between light stimulus and food delivery. The average ratio of on/off pressing frequency is plotted with a closed circles and bars of standard deviation. A reference line of 1 signifies the baseline expectation of subject performance when lever pressing when light is on is equal to lever pressing frequency when light is off. Ratio values greater than one are interpreted as preference for light stimulus and an increase in light preference is signified by an increase in ratio values over time. Subjects demonstrated a preference for the light stimulus, but did not develop an increase in preference over time.

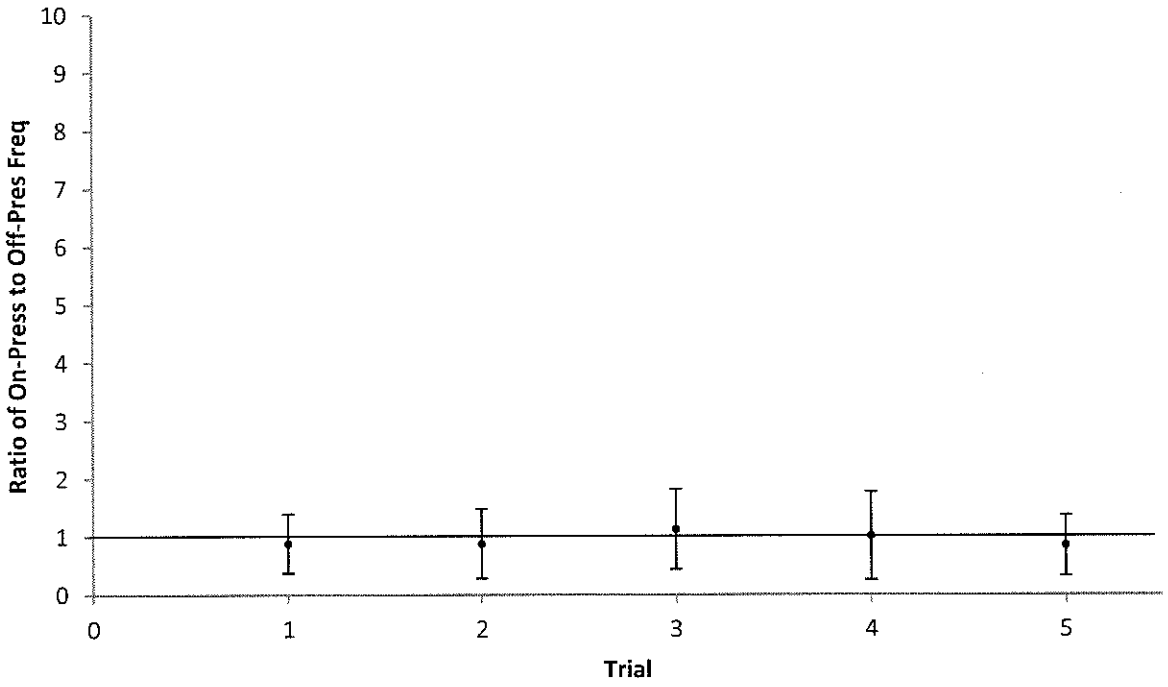

Figure 6. Operant Conditioning Results. Subjects were trained under the positive and negative reinforcement operant conditioning paradigm to train subjects to incorporate light stimulus dependence in lever response. The average ratio of light on/off pressing frequency of the subjects is plotted with closed circles and bars of standard deviation. The reference line signifies the baseline performance expectation, where frequency of lever pressing is equal when the light is on and off. Ratio values greater than 1 suggest subject preference for the light stimulus. Subjects did not demonstrate a preference for the light stimulus and did not develop a preference for the light stimulus over time.

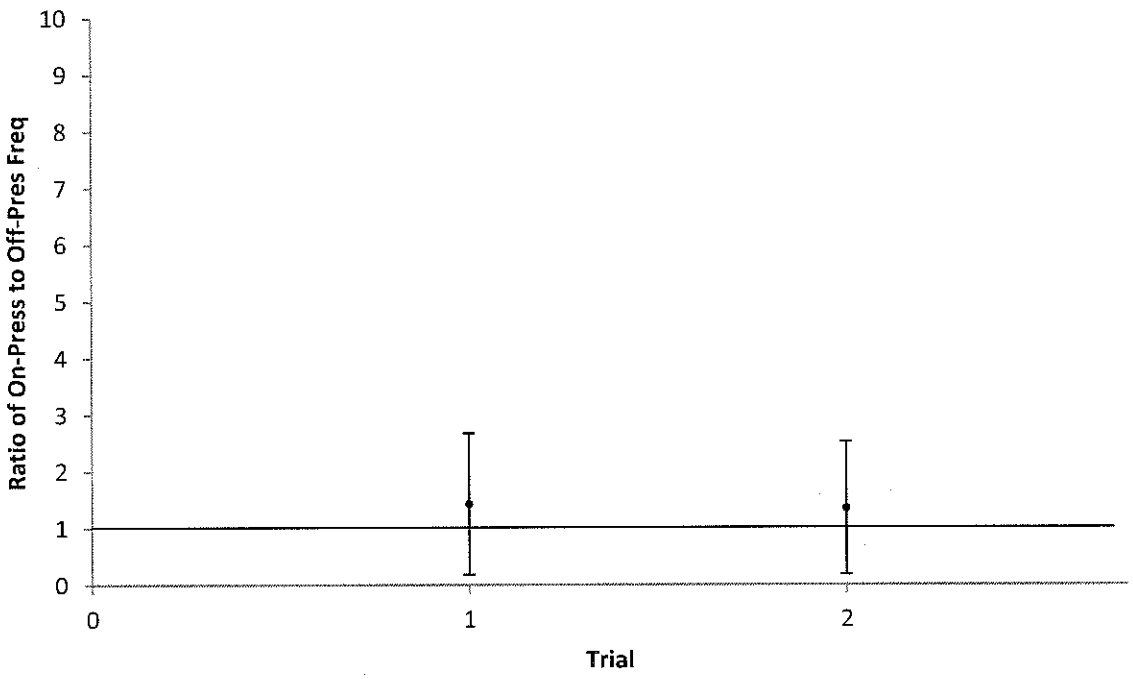

Figure 7. Two Wall Forced Choice Results. Subjects were trained under the forced choice paradigm with levers placed on two opposite walls to press the lever in response to the light stimulus. The average ratio of on/off pressing frequency is plotted with a closed circles and bars of standard deviation. The reference line represents the baseline expectation of ratio values. Values greater than baseline are interpreted as subject preference for the light stimulus. In these trials, subjects did not demonstrate light preference. Continuation of this phase was not pursued because of highly distracted subject behavior due to testing chamber setup.

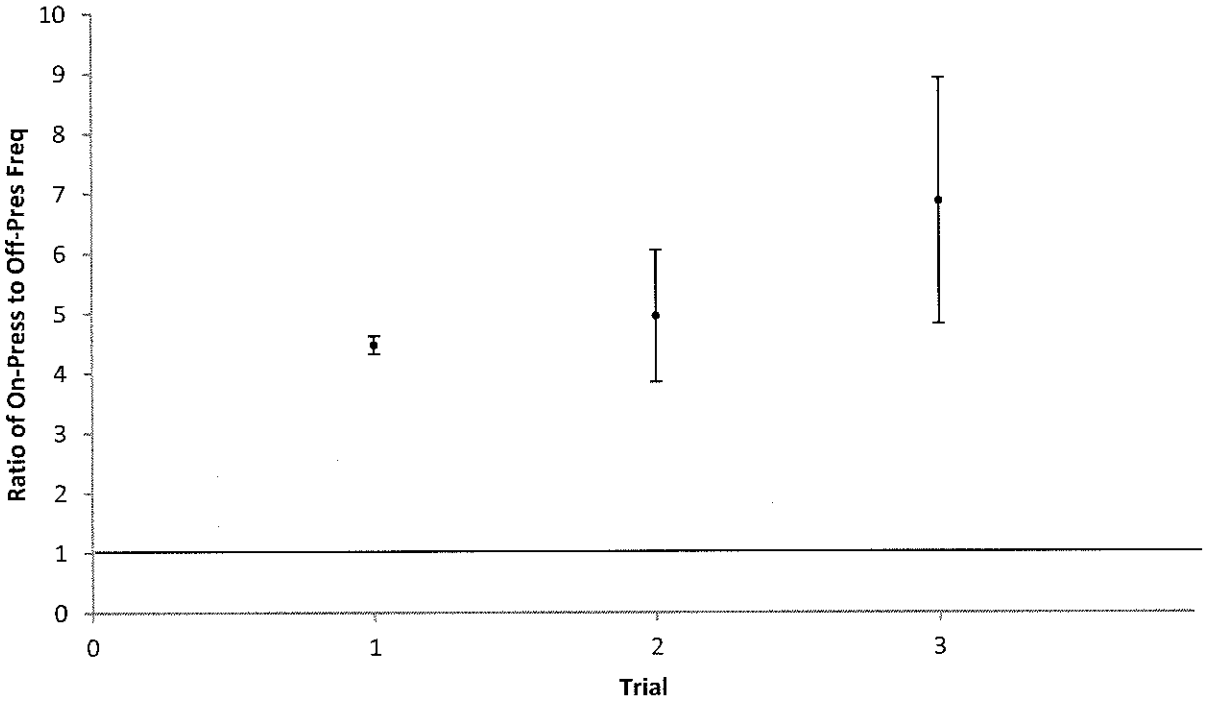

Figure 8. One Wall Forced Choice Results. Subjects were trained to press a lever in response to a visual stimulus under the forced choice paradigm with two levers placed on the same wall. The average ratio of light on/off pressing frequency is plotted for each trial as closed circles and bars of standard deviation. Ratio values greater than the baseline expectation (reference line) are interpreted as subject preference for the light stimulus. Subjects demonstrated preference for the light stimulus and increased in preference over time.

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FINAL APPROVAL

Certification of review and approval by the Institute Animal Care and Use Committee:

Name:

_____

IACUC Chair

PLEASE TYPE

I. GENERAL INFORMATION

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[1] All correspondence from the IACUC will be sent to the PI.

Grant/Project Title[3]: Assessing Visual Function Using Behavioral Training in the Rat Model

II. SPONSORSHIP OF PROJECT

☐ Funded ☐ Pending ☒ New Proposal ☒ Not Externally Funded

A. Funding Agency / Source of Funds: Eli Lilly Applied Life Science Center

B. Dates of Project: From: 1/17/13 To: 2/14/13

[2] Emergency Contact is the individual who will be contacted regarding an animal's health or disposition when morbidity requires action.
[3] NIH and RHIT require that the grant and protocol titles match. The information in this protocol must agree with your grant pages.

III PERSONNEL

Provide the following information for ALL INDIVIDUALS to be involved with study. This includes the Principal Investigator (PI), co-investigators, technicians, and specific students involved directly with the animal care and/or study procedures. All personnel listed must have completed the Animal Research Training Program (ARTP) by project start date. Please attach curriculum vita (CV) for PI and Co-PI(s).

Name	Position	Years of Experience With the:		Date Completed ARTP
		Species	Techniques	
Jameel Ahmed	**PI**	**8**	**0**	**4/2010**
Shannon Tieken	**Facility Manager/ Lab. Tech.**	**7**	**0**	**8/2012**
Patricia Bacala	**AB Undergraduate**	**0**	**0**	**11/2012**
Students	**Undergraduate Researchers/ Master's Students**			* **All students are required to have completed the ARTP before handling animals**

IV. RATIONALE FOR ANIMAL USE, RESEARCH ALTERNATIVES, AND REFERENCES CITED

[A] Specify animals to be used for the coming year. Enter the total number of animals to be used in each Pain Classification Column.

General Information						Pain Classification		
Genus	Species	Common Name	Size	Age	Sex	Type A	Type B	Type C
Rattus	***norvegicus***	**Harlan Sprague Dawley rats**	**250-300 g**	**adult**	**male**	**6**		

Pain Classification Categories

Type A	**Type B**	**Type C**
(USDA C)	**(USDA D)**	**(USDA E)**
Pain or distress **will not** be induced; animals will be used only for collections, post-mortem dissections, injections or similar non-stressful procedures that only cause minor discomfort.	Pain or distress **could** be induced or there is a potential for the procedure to be painful, but will be relieved by appropriate drugs.	Pain or distress **will** be induced and will not be relieved; this category includes experiments where drug administration would interfere with the results.

[B] Explain your rationale for animal use. ***[The rationale should include reasons why non-animal models cannot be used.]***

The fundamental focus of the experiments described in this protocol is to explore the feasibility of behavioral conditioning to generate a desired response to visual stimuli in a mammal. Furthermore, these studies will employ both normal rats and rats that have a mutation that will lead to degeneration of their retinas. This will allow us to examine how loss of visual function is reflected in behavioral responses.

In the long run, the PI hopes to develop an animal model that can be used to study how mammals adjust to implantation of a visual neuroprosthetic device (note that we are not suggesting that we will use implants as part of the study proposed here). The behavioral studies discussed in this protocol would be employed in this project to see if animals that have a neuroprosthetic implant are able to use the information provided by that device. The outcome of these experiments will hopefully provide some baseline data for use in a grant application for further study into neuroprosthetic devices. Furthermore, this study should provide an excellent undergraduate research platform.

[C] Justify **the appropriateness of the species selected.** ***[The species selected should be the lowest possible on the phylogenetic scale.]***

Rats are a standard species for investigating behavioral responses and a large volume of research has been performed in retinal degeneration in the rat model. Current facilities and experience of the PI allow for proper care and handling of the rats with minimal adjustments.

[D] Justify the number of animals to be used. ***[The number of animals should be the minimum number required to obtain statistically valid results.]***

The number of animals to be required in the study is 6: 3 rats in which vision in not manipulated and 3 rats with retinal degeneration. Standards in experimental design call for at least 3 trials for statistically valid results to be obtained.

[E] Consideration of Alternatives for Pain Classifications B and C

If any procedures fall into Classifications B or C, causing more than momentary or slight pain or distress to the animals, describe your consideration of alternatives, including methods that (1) refine existing tests by minimizing animal distress, (2) reduce the number of animals necessary for the pain category, and your determination that alternatives are not available. Please also delineate the methods and sources used in your search for alternatives.

N/A

[F] Describe Pain Management

1. Describe Pain Management Procedure [Classification B]

N/A

2. Provide justification if no Pain Management Procedure(s) is used [Classification C]

N/A

[G] References Cited

1. Describe the literature review procedure used for this project.

We performed a search for behavioral conditioning of rats with visual stimulus in PubMed and followed up with references cited in the primary study.

2. List 2 or 3 literature references most directly related to the project.

1. Cleland, Gary G., and Graham CL Davey. "Autoshaping in the rat: The effects of localizable visual and auditory signals for food." *Journal of the experimental analysis of behavior* 40.1 (1983): 47.
Skinner, Burphus Frederic, C. B. Ferster, and Charles B. Ferster. *Schedules of reinforcement*. Copley Publishing Group, 1997.
LaVail, Matthew M. "Retinal Degeneration Rat Model Resource Availability of P23H and S334ter Mutant Rhodopsin Transgenic Rats and RCS Inbred and RCS Congenic Strains of Rats." University of California, San Francisco.

[H] Housing

1. Describe the primary housing for the animals. Include Building and Room #.

Animals will be housed in the vivarium in O109B. Animals will be housed individually or in pairs in 33x15x13 cm (L x W x H) cages.

2. Location of experiments.

M112 (Myers' Hall)

[I] Transportation

If animals will be transported, describe the methods, the containment, the route and elevator(s) to be utilized.

Animals will be transported in a small cage with a wire lid which will in turn be placed in an unsealed cardboard box for the trip to Myers' Hall. Animals will be transported via the 1st floor hallways in Olin, Hadley and Moench Halls.

V. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES

Briefly explain the experimental design and specify all animal procedures. This description should allow the IACUC to understand the experimental course of an animal from its entry

into the experiment to the endpoint of the study. Specifically address the following items for the Experimental Group(s) and Control Group(s), as applicable:

- **Injections or inoculations** (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules).
- **Blood withdrawals** (volume, frequency, withdrawal sites, and methodology).
- **Surgical procedures** (provide details of survival and non-survival surgical procedures in Section VI.).
- **Experimental timeline** (include timeframe and duration of each relevant activity of the project)
- **Radiation** (dosage and schedule).
- **Methods of restraint** (e.g., restraint chairs, collars, vests, harnesses, slings, etc.). Include how animals are restrained for routine procedures like blood withdrawals. Prolonged restraint must be justified with appropriate oversight to ensure it is minimally distressing. Describe any sedation, acclimation or training to be utilized.
- **Animal identification methods** (e.g., ear tags, tattoos, collar, cage card, implant, etc.).
- **Other procedures** (e.g., survival studies, tail biopsies, etc.).
- **Resultant effects**, if any, that the animals are expected to experience (e.g., pain or distress, ascites production, etc.).
- **Other potential stressors** (e.g., food or water deprivation, noxious stimuli, environmental stress) **and procedures to monitor and minimize distress.** If a study is Classification B, indicate any non-pharmaceutical methods to minimize pain and distress.
- **Experimental endpoint criteria** (e.g., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.
- **Veterinary care** (indicate desired plan of action in case of animal illness, e.g., initiate treatment, call investigator prior to initiating treatment, euthanize).

[A] Experimental Group(s)

Animal Procedures:

The goal of these experiments is to develop a test that can determine if rats are able to perceive a visual stimulus. To do this, rats will be conditioned to correlate a specific visual stimulus, for instance a flashing light of a given wavelength, with a food reward. Specifically, we will be doing this via a classical conditioning technique known as autoshaping. In early trials, rats will be placed in a testing box and a visual stimulus will

be presented. This stimulus will be paired with the presentation of a food reward. Eventually, the rat will start to respond behaviorally to the stimulus expecting a reward. By altering parameters of the stimulus (e.g. wavelength, intensity, duration), we can determine the limits of the rats visual system. It's possible we will extend the training to more complicated behaviors by extending the autoshaping paradigm, although in all cases positive rewards only will be used.

We will be developing the technique on normal-vision rats. The first two rats we would like to use are left over from a previous study. Once we have the technique worked out, we will extend our experiments to RDS rats which have a mutation that leads their retinas to degenerate. These animals will be obtained from a breeding facility which maintains colonies of several strains of RDS rats, we will not be doing any genetic modifications of rats here at Rose-Hulman. Note that this study does not require any surgeries, drug injections or noxious stimuli.

Patricia Bacala is the undergraduate who will be the primary caretaker of the rats and the principal researcher. This project is part of Patricia's Senior Thesis project. Patricia has already been involved in caring for rats and has passed the CITI animal training program. She will be responsible for developing the training protocol and for running the training sessions for the rats. We expect that during early training sessions, rats may be apprehensive and Patricia will use protective gloves while handling them. Rats will likely become quickly accustomed to being handled and to the training process and we feel that there is little risk of animals causing injury to students or themselves.

Experimental Timeline:

Development of training techniques and acquisition of data from normal vision rats will occur during the Winter and Spring Terms of AY2012-13. Depending on availability, RDS rats will be trained during the Fall quarter of 2013-14. This timeframe may be adjusted depending on how quickly the rats respond to training. At the end of the study the animals will be euthanized by the PI.

Animal Identification Methods:

Animals will be identified using cage cards and by marking individual animals with marker.

Other potential stressors and procedures:

Some minor (nonphysical) stress may be involved in the early stages of growing accustomed to the new handler.

Food and/or water will be withheld 12 hours prior to the training sessions to encourage desired behavior with the appropriate reward.

Testing will occur in M112 and rats will be transported from the vivarium for the training. Prior to the successive approximation training events, the rats will be transported to M112 to get used to the transport process and minimize stress during the training time.

Due to the length of this study and a history of rats gaining weight while at our facility, an exercise program for the rats is proposed. Based on other studies in which swimming is applied, the proposed regimen will exercise each rat for 15 minute periods three times each week. A container approximately 10 in wide X 20 in long and 12 in deep will be filled 2/3 full with tap water and up to 2 rats will be allowed to swim. After 15 minutes, the rats will be removed from the water, dried and returned to their cage.

Procedures to monitor and minimize distress:

Animals will be housed individually or in pairs and will be inspected daily for signs of distress.

Veterinary Care:

Veterinary Care will be provided by Dr. Holscher, the institute veterinarian. Dr. Holscher will be contacted when any health problems arise.

[B] Control Group(s)

The control group will consist of the rats without retinal degeneration.

VI. SURGERY

If proposed, complete the following:

[A] Identify and describe the surgical procedure(s) to be performed. Include preoperative procedures (e.g., fasting, analgesic loading), and monitoring and supportive care during surgery. Include the aseptic methods to be utilized.

N/A

[B] Who will perform surgery and what are their qualifications and/or experience?

N/A

[C] Where will surgery be performed and postoperative care provided (building and rooms)?

N/A

[D] If survival surgery, describe postoperative care required, frequency of observation, and identify the responsible individual(s). Include detection and management of postoperative complications during work hours, after hours, weekends and holidays.

N/A

[E] If non-survival surgery, describe how humane euthanasia is enacted and how death is determined.

N/A

[F] Are paralytic agents used during surgery? Yes ☐ or No ☒.
If yes, please describe how ventilation will be maintained and how pain will be assessed.

N/A

[G] Has major survival surgery been performed on any animal prior to being placed on this study? ***[Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation).]***
Yes ☐ or No ☒.

If yes, please explain:

N/A

[H] Will more than one major survival surgery be performed on an animal while on this study?
Yes ☐ or No ☒.

If yes, please justify:

N/A

VII. ANESTHETICS, ANALGESICS, SEDATIVES, TRANQUILIZERS, OR OTHER PHARMACOLOGIC AGENTS

List the anesthetics, analgesics, sedatives, tranquilizers, or other pharmacologic agents to be used. Include the name of the agent(s), the dosage, route and schedule of administration. If information is provided in Section IV above, please cross-reference. Describe tracking and security of controlled drugs (Drug Enforcement Agency requirements).

N/A

VIII. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY

Indicate the proposed method of euthanasia. If a chemical agent is used specify the dosage and route of administration. If the method(s) of euthanasia include those **not** recommended by the American Veterinarian Medical Association (AVMA) Panel Report on Euthanasia (e.g., decapitation or cervical dislocation without anesthesia), provide scientific justification why such methods must be used. Indicate the method of carcass or tissue disposal if not described in Section IX below.

At the end of the experiments, euthanasia will be carried out using CO_2 gas following Sevoflurane anesthesia. During euthanasia, the animals will be anesthetized in an induction chamber using Sevoflurane anesthesia (at least 4%). Once animals reach a state of unconsciousness, the Sevoflurane gas will be replaced with CO_2. Death will be confirmed by direct observation of the heart after a thoracotomy. After euthanasia, carcasses will be placed in a freezer in M112 or O109C until they are picked up with other biohazard waste.

IX. HAZARDOUS MATERIALS and BIOLOGICAL AGENTS

Are hazardous materials and/or biological agents (radioactive, hazardous chemicals, drugs, recombinant DNA, etc.) to be used in this project? **Yes** ☐ **or No** ☒

If yes, the use of hazardous materials and biological agents requires approval from the Office of Environmental Health and Safety. Attach documentation of approval for the use of these materials.

Approval must be obtained before any hazardous materials or biological agents are purchased.

Describe the practices and procedures required for the safe handling and disposal of animals and material associated with this study. Also describe methods for removal of all waste and, if applicable, the monitoring of exposure by the personnel involved with this protocol.

N/A

Additional safety considerations:

N/A

X. GENETIC MANIPULATIONS OF ANIMALS (Transgenic and Knockout)

Describe any phenotypic consequences of the genetic manipulations to the animals. Describe any special care or monitoring that the animals will require.

A strain of rats with known retinal degeneration will be used. These animals will be obtained from UCSF's **Retinal Degeneration Rat Model Resource, an NSF-funded resource which provides animals to investigators free of charge (other than shipping charges).** This resource provides multiple different strains of rats with known rates of retinal degeneration. **We will not be doing any genetic manipulations ourselves.** We also do not intend to breed animals at RHIT, and the risk of rats interacting with local wild populations is minimal.

XI. FIELD STUDIES

If animals in the wild will be used, describe how they will be observed, any interactions with the animals, whether the animals will be disturbed or affected, and any special procedures anticipated. Indicate if Federal permits are required and whether they have been obtained.

N/A

XII. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY

Identify other special circumstances or requirements not previously described in this document:

N/A

XIII. PRINCIPAL INVESTIGATOR CERTIFICATIONS

The undersigned, being the Principal Investigator in the research project described on the preceding pages of this document, hereby gives assurance that he/she will comply fully with Federal Law as set forth in the Animal Welfare Act; further that, if the research protocol described herein is approved by the Institute Animal Care and Use Committee, the investigator certifies:

1. I have attended the Animal Research Training Program required investigator training course.
2. I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. All individuals working on this proposal who are at risk are participating in the Institution's Occupational Health and Safety Program.
4. The individuals listed in Section III are authorized to conduct procedures involving animals under this proposal, have attended the institutionally required investigator training course, and received training in: the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns.
5. For all Pain Classification B and C protocols (see Section IV. [A]): I have reviewed the pertinent scientific literature and the sources and/or databases as noted in Section IV. G. and have found no valid alternative to any procedures described herein that may cause more than momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the IACUC before initiating any significant changes in this study.
7. I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and the IACUC.
8. I am familiar with and will comply with all pertinent institution policies, as well as all federal, state, and local regulations.

Principal Investigator:

Name: Jameel Ahmed Signature: _____ Date: _____

XIV. ADDITIONAL APPROVALS

Department Head of Principal Investigator:

Name: Jameel Ahmed Signature: _____ Date: _____

Department Head

Environmental Health and Safety:

(Required for all studies utilizing hazardous/biological materials)

Name: Jacob Campbell Signature: _____ Date: _____

Environmental Health and Safety Coordinator

Facility Manager certification of resource capability in the indicated facility to support the proposed study:

Name: Shannon M. Tieken Signature: _____ Date: _____

Facility Manager

Attending Veterinarian certification of review, resource capability in the indicated facility to support the proposed study, and consultation on proper use of anesthetics and pain relieving medications for any painful procedures:

Name: James Holscher Signature: _____ Date: _____

Doctor of Veterinary Medicine

Rat	Minute	1	2	3	4	5	1	2	3	4	5	6	7	8	9
35	1	1	0	1	2	1	1	0	0	1	9	4	10	11	14
	2	0	0	0	1	1	0	3	1	5	6	6	11	9	11
	3	2	2	0	1	2	1	0	0	5	11	6	13	9	12
	4	1	1	2	0	0	0	0	0	5	6	3	6	4	10
	5	2	2	1	2	3	0	0	0	9	3	9	5	11	11
	6	4	1	1	1	0	0	1	0	4	8	9	13	10	16
	7	1	3	2	2	1	0	0	0	9	11	14	16	4	11
	8	3	0	1	1	1	0	2	1	8	11	16	9	9	13
	9	0	3	1	2	2	2	1	0	4	12	6	9	10	15
	10	1	2	0	1	0	1	0	0	9	11	12	15	6	8
	11	1	4	2	0	1	0	1	1	9	5	13	8	7	11
	12	2	0	1	1	2	1	0	3	11	1	11	9	9	11
	13	1	2	1	2	0	0	0	0	12	3	12	8	5	12
	14	1	1	3	1	0	1	0	2	7	15	4	7	7	10
	15	0	1	1	1	1	0	1	1	10	8	7	4	9	9
	16	1	2	0	0	2	0	1	2	13	7	12	8	6	8
	17	2	0	0	2	0	0	0	4	8	10	8	4	4	6
	18	0	1	1	1	0	0	0	2	3	15	8	5	20	5
	19	1	2	1	1	1	0	0	4	11	9	9	4	12	6
	20	0	2	1	0	1	0	1	6	8	6	2	3	12	8
Total		24	29	20	22	19	7	11	27	151	167	171	167	174	207
36	1	2	1	2	3	0	2	2	2	6	6	17	14	3	11
	2	3	1	2	1	0	1	4	3	11	10	7	10	4	6
	3	1	3	1	2	1	1	2	3	11	11	6	14	4	12
	4	0	1	1	0	1	0	2	4	7	8	9	15	7	7
	5	0	1	3	0	2	2	1	3	11	2	13	13	6	16
	6	2	2	3	1	0	0	0	4	12	13	15	17	4	11
	7	2	1	1	1	2	1	1	2	7	11	11	21	5	10
	8	0	1	0	2	1	0	0	3	9	8	10	16	7	16
	9	2	2	0	2	0	1	1	4	9	10	7	12	9	13
	10	1	1	1	1	0	0	1	5	16	12	12	15	9	25
	11	1	1	1	2	1	1	1	3	10	12	8	12	6	9
	12	0	2	2	0	1	0	1	6	15	9	9	16	11	5
	13	0	0	2	0	3	1	0	5	12	9	9	15	6	16
	14	0	0	2	1	0	1	1	3	9	10	5	17	4	11
	15	0	0	0	1	1	0	0	3	4	11	8	20	1	10
	16	1	2	1	1	2	1	2	2	6	11	7	17	4	18
	17	1	0	1	0	2	0	2	1	8	9	8	10	5	17
	18	0	1	1	1	0	1	2	3	8	9	7	13	10	14
	19	2	1	0	1	1	2	1	3	8	10	13	15	2	6
	20	1	0	1	2	1	1	1	5	15	8	15	14	12	7
Total		19	21	25	22	19	16	25	67	194	189	196	296	119	240

Matlab code output of the number of lever counts per minute in baseline and autoshaping trials for subjects 35 and 36.

Rat		1 Total Marks	2 Total Marks	3 Total Marks	4 Total Marks
35	**1**	12	10	9	15
	2	4	13	7	12
	3	6	15	9	6
	4	10	13	12	15
	5	10	19	13	13
	6	9	16	7	8
	7	11	15	14	1
	8	7	8	11	11
	9	12	9	15	17
	10	11	7	16	7
	11	8	11	15	10
	12	10	3	13	4
	13	6	6	13	9
	14	10	10	9	8
	15	9	10	7	12
	16	9	11	11	8
	17	16	15	6	2
	18	11	16	15	7
	19	6	15	13	6
	20	10	12	14	8
Total		187	234	229	179

		1 Total Marks	2 Total Marks	3 Total Marks	4 Total Marks
36	**1**	8	13	17	11
	2	22	2	9	4
	3	9	18	12	11
	4	7	7	10	19
	5	8	12	12	14
	6	17	12	4	11
	7	11	12	15	8
	8	14	9	13	10
	9	14	12	12	9
	10	8	10	16	7
	11	8	14	12	17
	12	9	15	14	12
	13	7	15	12	5
	14	12	6	11	19
	15	15	10	16	9
	16	8	9	13	14
	17	9	8	11	13
	18	10	15	3	6
	19	13	16	8	7
	20	7	15	11	11
Total		216	230	231	217

Matlab code output for counts of lever presses during the Constant Light Stimulus trials

Rat		Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light
35	1	7	5	0.19	0.06	3.27	4	13	9	0.35	0.12	2.94	4	11	5	0.19	0.18	1.09	4	10	5	0.19	0.15	1.31	4
	2	13	9	0.28	0.15	1.90	5	11	7	0.22	0.15	1.48	5	11	6	0.18	0.18	1.02	5	14	7	0.22	0.25	0.85	5
	3	19	10	0.38	0.26	1.45	4	11	5	0.19	0.18	1.09	4	20	9	0.35	0.32	1.07	4	12	7	0.27	0.15	1.83	4
	4	22	13	0.40	0.33	1.22	5	13	7	0.22	0.22	0.99	5	24	16	0.49	0.29	1.69	5	10	8	0.25	0.07	3.38	5
	5	13	8	0.25	0.18	1.35	5	8	1	0.03	0.25	0.12	5	9	4	0.12	0.18	0.68	5	10	6	0.18	0.15	1.27	5
	6	10	6	0.23	0.12	1.96	4	11	7	0.27	0.12	2.29	4	17	10	0.38	0.21	1.87	4	5	4	0.15	0.03	5.23	4
	7	15	11	0.34	0.15	2.33	5	16	8	0.25	0.29	0.85	5	18	12	0.37	0.22	1.69	5	12	6	0.18	0.22	0.85	5
	8	15	9	0.35	0.18	1.96	4	14	9	0.35	0.15	2.35	4	13	9	0.35	0.12	2.94	4	11	6	0.23	0.15	1.57	4
	9	23	15	0.46	0.29	1.59	5	16	10	0.31	0.22	1.41	5	8	5	0.15	0.11	1.41	5	13	11	0.34	0.07	4.85	5
	10	16	8	0.25	0.29	0.85	5	17	6	0.18	0.40	0.46	5	16	11	0.34	0.18	1.86	5	9	5	0.15	0.15	1.06	5
	11	8	5	0.19	0.09	2.18	4	22	13	0.50	0.26	1.89	4	13	7	0.27	0.18	1.53	4	6	4	0.15	0.06	2.62	4
	12	14	9	0.28	0.18	1.52	5	10	5	0.15	0.18	0.85	5	10	6	0.18	0.15	1.27	5	12	6	0.18	0.22	0.85	5
	13	21	9	0.28	0.44	0.63	5	15	8	0.25	0.25	0.97	5	11	7	0.22	0.15	1.48	5	12	5	0.15	0.25	0.60	5
	14	11	8	0.31	0.09	3.49	4	16	7	0.27	0.26	1.02	4	13	7	0.27	0.18	1.53	4	12	6	0.23	0.18	1.31	4
	15	5	3	0.09	0.07	1.27	5	21	10	0.31	0.40	0.77	5	9	5	0.15	0.15	1.06	5	10	6	0.18	0.15	1.27	5
	16	8	4	0.15	0.12	1.31	4	16	10	0.38	0.18	2.18	4	4	2	0.08	0.06	1.31	4	15	7	0.27	0.24	1.14	4
	17	13	11	0.34	0.07	4.65	5	11	7	0.22	0.15	1.48	5	13	6	0.18	0.25	0.73	5	6	3	0.09	0.11	0.85	5
	18	10	5	0.15	0.18	0.85	5	15	8	0.25	0.25	0.97	5	3	1	0.03	0.07	0.42	5	7	4	0.12	0.11	1.13	5
	19	11	8	0.31	0.09	3.49	4	16	11	0.42	0.15	2.88	4	11	9	0.35	0.06	5.89	4	10	6	0.23	0.12	1.96	4
	20	12	7	0.22	0.18	1.18	5	16	12	0.37	0.15	2.54	5	12	10	0.31	0.07	4.23	5	12	7	0.22	0.18	1.18	5
		266		0.27		1.92		288		0.27		1.48		246		0.25		1.74		208		0.20		1.75	
		Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light	Total Ma	Matched	Frequen	Frequen	Ratio	Light
		5	4	0.15	0.03	5.23	4	5	3	0.12	0.06	1.96	4	9	4	0.15	0.15	1.05	4	6	3	0.12	0.09	1.31	4
		11	4	0.12	0.25	0.48	5	9	4	0.12	0.18	0.68	5	10	5	0.15	0.18	0.85	5	15	6	0.18	0.33	0.56	5
		12	7	0.27	0.15	1.83	4	7	4	0.15	0.09	1.74	4	9	4	0.15	0.15	1.05	4	11	7	0.27	0.12	2.29	4
		13	7	0.22	0.22	0.99	5	9	7	0.22	0.07	2.96	5	8	6	0.18	0.07	2.54	5	10	5	0.15	0.18	0.85	5
		5	1	0.03	0.15	0.21	5	7	1	0.03	0.22	0.14	5	15	7	0.22	0.29	0.74	5	12	7	0.22	0.18	1.18	5
		10	7	0.27	0.09	3.05	4	14	6	0.23	0.24	0.98	4	11	5	0.19	0.18	1.09	4	11	5	0.19	0.18	1.09	4
		12	7	0.22	0.18	1.18	5	14	5	0.15	0.33	0.47	5	10	2	0.06	0.29	0.21	5	16	7	0.22	0.33	0.66	5
		13	7	0.27	0.18	1.53	4	7	4	0.15	0.09	1.74	4	7	4	0.15	0.09	1.74	4	5	2	0.08	0.09	0.87	4
		6	4	0.12	0.07	1.69	5	14	7	0.22	0.25	0.85	5	8	5	0.15	0.11	1.41	5	12	7	0.22	0.18	1.18	5
		7	6	0.18	0.04	5.08	5	11	4	0.12	0.25	0.48	5	11	5	0.15	0.22	0.71	5	16	10	0.31	0.22	1.41	5
		16	10	0.38	0.18	2.18	4	12	5	0.19	0.21	0.93	4	11	6	0.23	0.15	1.57	4	15	9	0.35	0.18	1.96	4
		10	6	0.18	0.15	1.27	5	10	4	0.12	0.22	0.56	5	16	8	0.25	0.29	0.85	5	10	5	0.15	0.18	0.85	5
		16	8	0.25	0.29	0.85	5	9	6	0.18	0.11	1.69	5	16	7	0.22	0.33	0.66	5	16	9	0.28	0.25	1.09	5
		19	11	0.42	0.24	1.80	4	12	5	0.19	0.21	0.93	4	18	8	0.31	0.29	1.05	4	16	11	0.42	0.15	2.88	4
		16	9	0.28	0.25	1.09	5	13	8	0.25	0.18	1.35	5	19	10	0.31	0.33	0.94	5	7	4	0.12	0.11	1.13	5
		11	7	0.27	0.12	2.29	4	6	3	0.12	0.09	1.31	4	18	9	0.35	0.26	1.31	4	10	5	0.19	0.15	1.31	4
		9	6	0.18	0.11	1.69	5	6	2	0.06	0.15	0.42	5	15	8	0.25	0.25	0.97	5	10	6	0.18	0.15	1.27	5
		12	8	0.25	0.15	1.69	5	8	3	0.09	0.18	0.51	5	5	4	0.12	0.04	3.38	5	17	8	0.25	0.33	0.75	5
		17	9	0.35	0.24	1.47	4	7	5	0.19	0.06	3.27	4	10	8	0.31	0.06	5.23	4	13	8	0.31	0.15	2.09	4
		7	5	0.15	0.07	2.12	5	12	6	0.18	0.22	0.85	5	12	4	0.12	0.29	0.42	5	7	3	0.09	0.15	0.63	5
		227		0.23		1.89		192		0.16		1.19		238		0.20		1.39		235		0.21		1.27	

Matlab code output of Autoshaping with Light trials for Subject 35. Trial order moves from left to right for each row.

		Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light
36	1	9	6	0.23	0.09	2.62	4	12	8	0.31	0.12	2.62	4	13	9	0.35	0.12	2.94	4	8	5	0.28	0.07	3.89	4
	2	9	5	0.15	0.15	1.06	5	13	6	0.18	0.25	0.73	5	7	5	0.15	0.07	2.12	5	15	8	0.36	0.19	1.90	5
	3	8	5	0.19	0.09	2.18	4	10	6	0.23	0.12	1.96	4	12	9	0.35	0.09	3.92	4	14	8	0.44	0.14	3.11	4
	4	13	11	0.34	0.07	4.65	5	17	9	0.28	0.29	0.95	5	9	4	0.12	0.18	0.68	5	17	10	0.44	0.19	2.38	5
	5	9	4	0.12	0.18	0.68	5	17	8	0.25	0.33	0.75	5	17	8	0.25	0.33	0.75	5	10	3	0.13	0.19	0.71	5
	6	5	4	0.15	0.03	5.23	4	22	13	0.50	0.26	1.89	4	11	8	0.31	0.09	3.49	4	12	9	0.50	0.07	7.00	4
	7	8	5	0.15	0.11	1.41	5	9	4	0.12	0.18	0.68	5	18	12	0.37	0.22	1.69	5	9	4	0.18	0.13	1.33	5
	8	12	7	0.27	0.15	1.83	4	10	5	0.19	0.15	1.31	4	15	9	0.35	0.18	1.96	4	10	3	0.17	0.17	1.00	4
	9	8	5	0.15	0.11	1.41	5	10	5	0.15	0.18	0.85	5	16	12	0.37	0.15	2.54	5	15	11	0.43	0.11	4.58	5
	10	8	4	0.12	0.15	0.85	5	13	8	0.25	0.18	1.35	5	10	5	0.15	0.18	0.85	5	12	9	0.40	0.08	5.00	5
	11	10	9	0.35	0.03	11.77	4	19	12	0.46	0.21	2.24	4	10	5	0.19	0.15	1.31	4	13	7	0.39	0.14	2.72	4
	12	6	6	0.18	0.00	12.00	5	16	9	0.28	0.25	1.09	5	15	10	0.31	0.18	1.69	5	14	11	0.49	0.08	6.11	5
	13	15	7	0.22	0.29	0.74	5	19	14	0.43	0.18	2.37	5	20	11	0.34	0.33	1.03	5	9	5	0.22	0.11	2.08	5
	14	12	7	0.27	0.15	1.83	4	14	10	0.38	0.12	3.27	4	10	7	0.27	0.09	3.05	4	9	5	0.28	0.10	2.92	4
	15	9	6	0.18	0.11	1.69	5	9	5	0.15	0.15	1.06	5	11	5	0.15	0.22	0.71	5	3	1	0.04	0.05	0.83	5
	16	8	4	0.15	0.12	1.31	4	13	8	0.31	0.15	2.09	4	22	8	0.31	0.41	0.75	4	1	1	0.06	0.00	7.00	4
	17	11	9	0.28	0.07	3.81	5	15	12	0.37	0.11	3.38	5	16	10	0.31	0.22	1.41	5	11	8	0.36	0.08	4.44	5
	18	7	5	0.15	0.07	2.12	5	8	4	0.12	0.15	0.85	5	19	12	0.37	0.25	1.46	5	12	6	0.27	0.16	1.67	5
	19	8	5	0.19	0.09	2.18	4	9	4	0.15	0.15	1.05	4	13	9	0.35	0.12	2.94	4	17	9	0.50	0.19	2.63	4
	20	11	5	0.15	0.22	0.71	5	9	8	0.25	0.04	6.77	5	15	7	0.22	0.29	0.74	5	9	5	0.22	0.11	2.08	5
Total		186		0.20		3.00		264		0.27		1.86		279		0.28		1.80		220		0.31		3.17	
		Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light	Total Ma	Matched	Frequency On	Frequency Off	Ratio	Light
		13	9	0.35	0.12	2.94	4	16	7	0.27	0.26	1.02	4	18	11	0.42	0.21	2.05	4	12	7	0.27	0.15	1.83	4
		10	5	0.15	0.18	0.85	5	15	8	0.25	0.25	0.97	5	14	6	0.18	0.29	0.63	5	10	5	0.15	0.18	0.85	5
		12	4	0.15	0.24	0.65	4	14	7	0.27	0.21	1.31	4	16	10	0.38	0.18	2.18	4	12	3	0.12	0.26	0.44	4
		10	8	0.25	0.07	3.38	5	10	6	0.18	0.15	1.27	5	20	13	0.40	0.25	1.57	5	13	7	0.22	0.22	0.93	5
		16	10	0.31	0.22	1.41	5	21	12	0.37	0.33	1.13	5	10	4	0.12	0.22	0.56	5	17	12	0.37	0.18	2.03	5
		10	6	0.23	0.12	1.96	4	19	11	0.42	0.24	1.80	4	8	4	0.15	0.12	1.31	4	16	10	0.38	0.18	2.18	4
		12	7	0.22	0.18	1.18	5	9	5	0.15	0.15	1.06	5	16	7	0.22	0.33	0.66	5	13	9	0.28	0.15	1.90	5
		10	7	0.27	0.09	3.05	4	6	3	0.12	0.09	1.31	4	14	8	0.31	0.18	1.74	4	15	8	0.31	0.21	1.49	4
		13	9	0.28	0.15	1.90	5	12	7	0.22	0.18	1.18	5	13	9	0.28	0.15	1.90	5	15	9	0.28	0.22	1.27	5
		22	13	0.40	0.33	1.22	5	13	5	0.15	0.29	0.53	5	15	10	0.31	0.18	1.69	5	12	6	0.18	0.22	0.85	5
		10	6	0.23	0.12	1.96	4	8	7	0.27	0.03	9.15	4	18	10	0.38	0.24	1.63	4	10	6	0.23	0.12	1.96	4
		8	4	0.12	0.15	0.85	5	13	8	0.25	0.18	1.35	5	17	11	0.34	0.22	1.55	5	11	6	0.18	0.18	1.02	5
		15	8	0.25	0.25	0.97	5	13	8	0.25	0.18	1.35	5	14	7	0.22	0.25	0.85	5	18	11	0.34	0.25	1.33	5
		13	9	0.35	0.12	2.94	4	12	7	0.27	0.15	1.83	4	21	15	0.58	0.18	3.27	4	13	8	0.31	0.15	2.09	4
		11	7	0.22	0.15	1.48	5	16	10	0.31	0.22	1.41	5	17	11	0.34	0.22	1.55	5	12	4	0.12	0.29	0.42	5
		8	4	0.15	0.12	1.31	4	8	6	0.23	0.06	3.92	4	13	7	0.27	0.18	1.53	4	15	7	0.27	0.24	1.14	4
		14	11	0.34	0.11	3.10	5	12	8	0.25	0.15	1.69	5	12	8	0.25	0.15	1.69	5	6	5	0.15	0.04	4.23	5
		9	6	0.18	0.11	1.69	5	17	7	0.22	0.36	0.59	5	21	13	0.40	0.29	1.38	5	14	7	0.22	0.25	0.85	5
		15	8	0.31	0.21	1.49	4	13	10	0.38	0.09	4.36	4	18	12	0.46	0.18	2.62	4	7	5	0.19	0.06	3.27	4
		9	4	0.12	0.18	0.68	5	12	6	0.18	0.22	0.85	5	17	11	0.34	0.22	1.55	5	6	4	0.12	0.07	1.69	5
		240		0.24		1.75		259		0.25		1.90		312		0.32		1.60		247		0.23		1.59	

Matlab code output of Autoshaping with Light trials for Subject 36. Trial order moves from left to right for each row. Highlighted cells indicate ratio values replaced usin method described earlier

Rat		Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Marks	Matched	Frequen	Frequen	Ratio	Light
35	1	4	1	0.11	0.06	1.89	2	18	2	0.22	0.31	0.71	2	8	1	0.11	0.14	0.81	2	18	3	0.22	0.32	0.69	3	9	0	0.00	0.18	0.00	2
	2	8	1	0.22	0.13	1.76	1	16	2	0.15	0.30	0.49	3	16	2	0.15	0.30	0.49	3	17	6	0.44	0.24	1.88	3	21	7	0.39	0.33	1.17	4
	3	5	1	0.11	0.09	1.42	2	12	1	0.07	0.24	0.31	3	12	4	0.44	0.16	2.83	2	13	3	0.67	0.18	3.70	1	11	3	0.17	0.19	0.88	4
	4	10	1	0.11	0.18	0.63	2	14	2	0.15	0.26	0.57	3	21	5	0.37	0.34	1.08	3	12	1	0.22	0.20	1.12	1	18	4	0.30	0.30	0.98	3
	5	11	4	0.30	0.15	1.97	3	16	2	0.15	0.30	0.49	3	13	1	0.11	0.24	0.47	2	12	2	0.22	0.20	1.13	2	12	2	0.15	0.22	0.69	3
	6	13	1	0.07	0.26	0.29	3	14	3	0.17	0.26	0.64	4	11	2	0.22	0.18	1.26	2	20	2	0.15	0.39	0.38	3	16	7	0.39	0.21	1.81	4
	7	10	0	0.00	0.18	0.00	1	16	3	0.33	0.25	1.31	2	19	1	0.22	0.32	0.69	1	14	4	0.44	0.20	2.27	2	19	1	0.11	0.35	0.31	2
	8	13	3	0.22	0.22	1.03	3	14	2	0.11	0.29	0.39	4	10	2	0.22	0.16	1.42	2	19	0	0.00	0.37	0.00	2	21	5	0.28	0.38	0.73	4
	9	11	3	0.33	0.16	2.13	2	16	3	0.33	0.25	1.31	2	12	5	0.37	0.15	2.46	3	17	3	0.33	0.27	1.21	2	19	5	0.37	0.30	1.23	3
	10	14	3	0.22	0.24	0.94	3	8	2	0.15	0.13	1.15	3	13	3	0.33	0.20	1.70	2	16	1	0.11	0.29	0.38	2	19	3	0.22	0.34	0.65	3
	11	9	2	0.15	0.15	0.98	3	12	2	0.15	0.22	0.69	3	13	2	0.22	0.22	1.03	2	20	3	0.67	0.31	2.18	1	17	7	0.52	0.22	2.41	3
	12	12	2	0.22	0.20	1.13	2	10	4	0.30	0.13	2.30	3	18	3	0.33	0.29	1.13	2	20	1	0.22	0.34	0.65	1	12	2	0.11	0.24	0.47	4
	13	13	1	0.07	0.26	0.29	3	10	1	0.22	0.16	1.37	1	10	2	0.22	0.16	1.42	2	17	0	0.00	0.33	0.00	2	9	2	0.11	0.17	0.67	4
	14	11	3	0.13	0.21	0.63	5	8	2	0.15	0.13	1.15	3	12	3	0.33	0.18	1.89	2	11	0	0.00	0.22	0.00	2	18	3	0.17	0.36	0.47	4
	15	10	3	0.17	0.17	1.00	4	12	3	0.22	0.19	1.15	3	15	3	0.22	0.26	0.86	3	13	1	0.07	0.26	0.29	3	15	2	0.11	0.31	0.36	4
	16	11	1	0.07	0.22	0.34	3	10	2	0.15	0.17	0.86	3	22	2	0.22	0.39	0.57	2	18	3	0.33	0.29	1.13	2	18	6	0.33	0.29	1.17	4
	17	9	0	0.00	0.21	0.00	4	16	3	0.22	0.28	0.79	3	16	3	0.33	0.25	1.31	2	16	6	0.33	0.24	1.40	4	8	1	0.11	0.14	0.81	2
	18	15	1	0.11	0.27	0.40	2	8	0	0.00	0.16	0.00	2	18	5	0.56	0.25	2.18	2	12	2	0.15	0.22	0.69	3	17	4	0.22	0.31	0.72	4
	19	14	2	0.22	0.24	0.94	2	8	2	0.22	0.12	1.89	2	19	2	0.22	0.33	0.67	2	18	4	0.44	0.27	1.62	2	17	2	0.22	0.29	0.76	2
	20	16	5	0.28	0.24	1.17	4	6	1	0.07	0.11	0.69	3	18	5	0.37	0.28	1.32	3	14	1	0.22	0.23	0.95	1	11	3	0.17	0.19	0.88	4
Total		216	38	0.16		0.95	2.7	244				0.91		296		0.28		1.28	2.2	317	46	0.26		1.08	2.1	307	69	0.22		0.86	3.35

Rat		Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light	Total Marks	Matched	Frequen	Frequen	Ratio	Light
36	1	14	1	0.07	0.28	0.26	3	3	1	0.11	0.04	0.00	2	12	0	0.00	0.24	0.00	2	17	1	0.11	0.31	0.35	2	10	0	0.00	0.20	0.00	2
	2	16	4	0.30	0.26	1.15	3	3	1	0.07	0.04	1.72	3	14	1	0.11	0.25	0.44	2	15	1	0.07	0.30	0.25	3	13	4	0.30	0.19	1.53	3
	3	18	2	0.15	0.34	0.43	3	9	3	0.22	0.13	1.72	3	11	2	0.11	0.21	0.52	4	14	1	0.07	0.28	0.26	3	17	5	0.37	0.26	1.44	3
	4	14	3	0.17	0.26	0.64	4	18	2	0.15	0.34	0.43	3	12	1	0.11	0.22	0.52	2	10	2	0.22	0.16	1.42	2	16	3	0.17	0.31	0.54	4
	5	17	2	0.22	0.29	0.76	2	6	2	0.22	0.08	2.83	2	9	3	0.17	0.14	1.17	4	11	2	0.22	0.18	1.26	2	18	5	0.28	0.31	0.90	4
	6	15	4	0.22	0.26	0.85	4	15	2	0.11	0.31	0.36	4	12	1	0.11	0.22	0.52	2	9	2	0.15	0.15	0.98	3	10	3	0.22	0.15	1.48	3
	7	11	3	0.22	0.17	1.29	3	9	1	0.11	0.16	0.71	2	20	3	0.67	0.31	2.18	1	14	2	0.22	0.24	0.94	2	9	2	0.11	0.17	0.67	4
	8	10	2	0.15	0.17	0.86	3	15	3	0.22	0.26	0.86	3	10	2	0.15	0.17	0.86	3	11	3	0.33	0.16	2.13	2	8	3	0.17	0.12	1.40	4
	9	15	4	0.30	0.24	1.25	3	12	2	0.22	0.20	1.13	2	10	3	0.17	0.17	1.00	4	8	0	0.00	0.16	0.00	2	12	1	0.07	0.24	0.31	3
	10	12	4	0.22	0.19	1.17	4	6	1	0.11	0.10	1.13	2	11	4	0.30	0.15	1.97	3	6	1	0.07	0.11	0.69	3	14	3	0.17	0.26	0.64	4
	11	13	2	0.11	0.35	0.42	4	15	2	0.11	0.31	0.36	4	16	1	0.22	0.27	0.82	1	17	3	0.33	0.27	1.21	2	9	1	0.06	0.19	0.29	4
	12	10	1	0.07	0.19	0.38	3	9	1	0.06	0.19	0.29	4	14	3	0.17	0.26	0.64	4	11	1	0.11	0.20	0.57	2	18	4	0.22	0.33	0.67	4
	13	8	3	0.17	0.12	1.40	4	18	3	0.22	0.32	0.69	3	14	4	0.22	0.24	0.93	4	15	3	0.33	0.24	1.42	2	12	2	0.15	0.22	0.69	3
	14	14	4	0.22	0.24	0.93	4	15	3	0.22	0.26	0.86	3	13	6	0.44	0.15	2.95	3	12	2	0.22	0.20	1.13	2	17	3	0.17	0.33	0.50	4
	15	9	2	0.15	0.15	0.98	3	19	3	0.22	0.32	0.69	3	12	2	0.11	0.24	0.47	4	12	2	0.15	0.22	0.69	3	1	1	0.07	0.00	2.00	3
	16	12	2	0.15	0.22	0.69	3	12	1	0.06	0.26	0.21	4	15	3	0.17	0.29	0.58	4	13	3	0.22	0.22	1.03	3	12	2	0.15	0.22	0.69	3
	17	11	1	0.07	0.22	0.34	3	15	2	0.15	0.28	0.53	3	15	4	0.22	0.26	0.85	4	13	3	0.17	0.24	0.70	4	12	3	0.22	0.19	1.15	3
	18	12	3	0.22	0.19	1.15	3	9	2	0.15	0.15	0.98	3	12	3	0.13	0.24	0.56	5	9	2	0.11	0.17	0.67	4	14	1	0.07	0.28	0.26	3
	19	16	3	0.22	0.28	0.79	3	12	1	0.07	0.24	0.31	3	14	2	0.22	0.24	0.94	2	10	2	0.15	0.17	0.86	3	17	3	0.17	0.33	0.50	4
	20	12	2	0.15	0.22	0.69	3	12	2	0.22	0.20	1.13	2	12	5	0.28	0.17	1.67	4	13	5	0.37	0.17	2.15	3	18	3	0.33	0.28	1.13	2
Total		259		0.18		0.82	3.25	231				0.85		259		0.20		0.98	3.1	240		0.18		0.94	2.6	257		0.17		0.84	3.35

Matlab code output of Operant Conditioning trials for subjects 35 and 36. Trial order moves from left to right for each row. Highlighted cell indicates value replaced to have 'true' ratio for analysis.

Rat	Baselin	Total Ma	Total Mi	Total Mar	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light
35	1	0	0	2	1	0	0.00	0.02	0.00	1	3	0	0.00	0.06	0.00	2
	2	0	0	1	2	0	0.00	0.04	0.00	1	2	1	0.07	0.02	3.44	3
	3	1	1	0	3	0	0.00	0.05	0.00	1	9	0	0.00	0.16	0.00	1
	4	0	0	1	2	1	0.11	0.02	5.67	2	4	0	0.00	0.07	0.00	1
	5	1	1	1	8	0	0.00	0.14	0.00	1	7	3	0.33	0.08	4.25	2
	6	1	1	2	7	2	0.22	0.10	2.27	2	2	0	0.00	0.04	0.00	1
	7	2	1	0	3	1	0.22	0.04	6.17	1	9	1	0.11	0.16	0.71	2
	8	0	2	0	5	0	0.00	0.09	0.00	1	2	1	0.11	0.02	5.67	2
	9	0	1	3	6	2	0.44	0.07	6.17	1	8	2	0.15	0.13	1.15	3
	10	0	0	2	4	2	0.15	0.04	3.44	3	7	1	0.11	0.12	0.94	2
	11	0	1	2	6	0	0.00	0.11	0.00	1	5	0	0.00	0.09	0.00	1
	12	1	0	1	6	1	0.11	0.10	1.13	2	6	0	0.00	0.11	0.00	1
	13	2	1	1	2	1	0.07	0.02	3.44	3	9	2	0.22	0.14	1.62	2
	14	2	0	0	2	1	0.22	0.02	12.33	1	5	2	0.22	0.06	3.78	2
	15	1	2	0	5	1	0.11	0.08	1.42	2	2	1	0.11	0.02	5.67	2
	16	1	1	1	6	2	0.15	0.09	1.72	3	8	2	0.22	0.12	1.89	2
	17	1	0	1	5	2	0.22	0.06	3.78	2	5	2	0.22	0.06	3.78	2
	18	0	1	2	4	1	0.22	0.05	4.11	1	6	0	0.00	0.12	0.00	2
	19	0	2	2	5	1	0.11	0.08	1.42	2	4	0	0.00	0.08	0.00	2
	20	1	0	1	5	1	0.22	0.07	3.08	1	4	0	0.00	0.07	0.00	1
Total		14	15	23	87		0.12963		2.8075	1.6	107		0.09444		1.64481	1.8

	Baseline															
		Total Ma	Total Mi	Total Mar	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mark	Matched	Frequen	Frequen	Ratio	Light
36	1	1	3	2	2	0	0.00	0.04	0.00	1	4	1	0.11	0.06	1.89	2
	2	5	2	2	1	1	0.22	0.00	7.00	1	4	1	0.11	0.06	1.89	2
	3	0	4	4	5	1	0.11	0.08	1.42	2	3	0	0.00	0.05	0.00	1
	4	4	1	1	6	2	0.15	0.09	1.72	3	6	1	0.22	0.09	2.47	1
	5	0	0	5	4	1	0.22	0.05	4.11	1	3	0	0.00	0.05	0.00	1
	6	0	3	3	4	0	0.00	0.07	0.00	1	5	0	0.00	0.09	0.00	1
	7	2	0	4	3	1	0.22	0.04	6.17	1	3	2	0.44	0.02	24.67	1
	8	5	2	0	0	0	0.00	0.00	0.00	1	4	1	0.22	0.05	4.11	1
	9	0	3	4	3	1	0.07	0.04	1.72	3	4	1	0.22	0.05	4.11	1
	10	2	2	2	2	1	0.11	0.02	5.67	2	3	2	0.44	0.02	24.67	1
	11	1	1	2	4	2	0.22	0.04	5.67	2	6	0	0.00	0.11	0.00	1
	12	0	3	1	3	1	0.07	0.04	1.72	3	3	1	0.22	0.04	6.17	1
	13	0	3	4	2	1	0.07	0.02	3.44	3	5	1	0.22	0.07	3.08	1
	14	2	3	3	3	2	0.15	0.02	6.89	3	4	0	0.00	0.07	0.00	1
	15	1	3	3	1	1	0.22	0.00	7.00	1	4	1	0.11	0.06	1.89	2
	16	2	4	2	1	1	0.11	0.00	7.00	2	5	1	0.22	0.07	3.08	1
	17	1	1	2	1	1	0.07	0.00	7.00	3	7	0	0.00	0.13	0.00	1
	18	1	3	3	3	2	0.15	0.02	6.89	3	5	0	0.00	0.09	0.00	1
	19	3	2	4	0	0	0.00	0.00	0.00	1	4	1	0.22	0.05	4.11	1
	20	4	4	5	0	0	0.00	0.00	0.00	1	3	1	0.22	0.04	6.17	1
Total		34	47	56	48		0.11		3.67	1.9	85		0.15		4.415	1.15

Matlab code output of wall C for the Two-Wall Forced Choice Paradigm for rats 35 and 36. Highlighted cells indicate replaced values in order to calculate 'true' ratios in statistical analysis.

Rat		Baseline Total Mar	Total Mar	Total Marks	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mar	Matched	Frequen	Frequen	Ratio	Light
35	1	7	0	0	4	0	0.00	0.07	0.00	1	4	1	0.22	0.05	4.11	1
	2	8	5	3	1	0	0.00	0.02	0.00	1	3	0	0.00	0.06	0.00	2
	3	1	3	3	3	2	0.22	0.02	11.33	2	1	0	0.00	0.02	0.00	3
	4	9	2	2	4	1	0.22	0.05	4.11	1	3	1	0.11	0.04	2.83	2
	5	7	4	4	0	0	0.00	0.00	0.00	1	2	1	0.11	0.02	5.67	2
	6	6	1	0	0	0	0.00	0.00	0.00	2	8	3	0.22	0.11	2.07	3
	7	2	1	1	1	0	0.00	0.02	0.00	2	0	0	0.00	0.00	0.00	2
	8	5	0	0	1	0	0.00	0.02	0.00	3	6	1	0.11	0.10	1.13	2
	9	1	1	3	5	1	0.22	0.07	3.08	1	1	0	0.00	0.02	0.00	1
	10	7	1	5	2	1	0.11	0.02	5.67	2	1	0	0.00	0.02	0.00	2
	11	12	2	0	3	1	0.07	0.04	1.72	3	4	1	0.11	0.06	1.89	2
	12	6	7	1	4	2	0.11	0.05	2.33	4	2	0	0.00	0.04	0.00	2
	13	7	4	1	2	0	0.00	0.04	0.00	1	1	0	0.00	0.02	0.00	2
	14	2	2	1	5	0	0.00	0.11	0.00	3	5	1	0.11	0.08	1.42	2
	15	6	3	2	5	1	0.22	0.07	3.08	1	3	1	0.11	0.04	2.83	2
	16	7	2	1	2	1	0.22	0.02	12.33	1	0	0	0.00	0.00	0.00	2
	17	6	2	2	6	0	0.00	0.11	0.00	1	1	1	0.07	0.00	0.00	3
	18	8	1	4	5	2	0.44	0.05	8.22	1	2	0	0.00	0.04	0.00	1
	19	2	2	1	5	1	0.22	0.07	3.08	1	1	0	0.00	0.02	0.00	2
	20	5	3	3	1	1	0.07	0.00	0.00	3	3	2	0.22	0.02	11.33	2
Total		114	46	37	59		0.11		2.75	1.75	51		0.07		1.66	2

		Baseline Total Mar	Total Mar	Total Marks	Total Marks	Matched	Frequen	Frequen	Ratio	Light	Total Mar	Matched	Frequen	Frequen	Ratio	Light
36	1	6	3	4	5	2	0.44	0.05	8.22	1	5	0	0.00	0.09	0.00	1
	2	4	4	4	6	2	0.22	0.08	2.83	2	9	1	0.22	0.14	1.54	1
	3	6	1	3	5	2	0.22	0.06	3.78	2	6	0	0.00	0.12	0.00	2
	4	0	4	5	4	1	0.11	0.06	1.89	2	1	1	0.22	0.00	0.00	1
	5	7	4	2	5	1	0.11	0.08	1.42	2	3	1	0.22	0.04	6.17	1
	6	4	2	2	6	2	0.22	0.08	2.83	2	5	1	0.22	0.07	3.08	1
	7	2	3	1	1	0	0.00	0.02	0.00	2	4	0	0.00	0.07	0.00	1
	8	3	7	4	3	1	0.22	0.04	6.17	1	6	2	0.44	0.07	6.17	1
	9	8	5	5	4	1	0.11	0.06	1.89	2	4	0	0.00	0.07	0.00	1
	10	2	4	3	7	3	0.33	0.08	4.25	2	6	0	0.00	0.11	0.00	1
	11	5	2	2	3	1	0.22	0.04	6.17	1	6	1	0.22	0.09	2.47	1
	12	10	6	1	4	2	0.11	0.05	2.33	4	9	2	0.44	0.13	3.52	1
	13	7	2	2	8	1	0.22	0.13	1.76	1	4	1	0.22	0.05	4.11	1
	14	3	3	3	6	0	0.00	0.12	0.00	2	8	1	0.22	0.13	1.76	1
	15	2	4	3	5	2	0.22	0.06	3.78	2	8	1	0.11	0.14	0.81	2
	16	6	4	2	0	0	0.00	0.00	0.00	1	7	2	0.44	0.09	4.93	1
	17	7	1	3	4	3	0.22	0.02	10.33	3	4	1	0.22	0.05	4.11	1
	18	5	3	3	5	3	0.22	0.04	5.17	3	5	2	0.15	0.06	2.30	3
	19	2	5	1	3	0	0.00	0.05	0.00	1	6	2	0.22	0.08	2.83	2
	20	8	2	4	2	1	0.11	0.02	5.67	2	8	0	0.00	0.14	0.00	1
Total		97	69	57	86		0.17		3.42	1.9	114		0.18		2.19	1.25

Matlab code output of wall A for the Two-Wall Forced Choice Paradigm for rats 35 and 36. Highlighted cells indicate replaced values in order to calculate 'true' ratios in statistical analysis.

Rat

35B	TotalMark	Matched	Frequenc	Frequency	Ratio	Light	TotalMark	Matched M	Frequenc	Frequenc	Ratio	Light	TotalMark	Matched	Frequenc	Frequency (	Ratio	Light
1	5	1	0.08	0.09	0.90	2	1	0	0.00	0.02	0.00	2	4	0	0.00	0.07	0.00	1
2	5	3	0.23	0.04	5.42	2	3	1	0.15	0.04	4.12	1	6	1	0.15	0.09	1.65	1
3	6	3	0.23	0.06	3.62	2	2	0	0.00	0.04	0.00	1	6	1	0.15	0.09	1.65	1
4	4	0	0.00	0.09	0.00	2	5	1	0.15	0.07	2.06	1	5	1	0.15	0.07	2.06	1
5	2	0	0.00	0.04	0.00	2	5	0	0.00	0.09	0.00	1	3	1	0.15	0.04	4.12	1
6	4	2	0.10	0.05	2.08	3	5	0	0.00	0.09	0.00	1	3	1	0.15	0.04	4.12	1
7	6	3	0.23	0.06	3.62	2	2	0	0.00	0.04	0.00	1	4	1	0.15	0.06	2.74	1
8	5	0	0.00	0.09	0.00	1	4	0	0.00	0.07	0.00	1	7	2	0.31	0.09	3.29	1
9	2	1	0.05	0.02	2.08	3	8	1	0.15	0.13	1.18	1	4	1	0.15	0.06	2.74	1
10	4	2	0.15	0.04	3.62	2	5	1	0.15	0.07	2.06	1	3	0	0.00	0.06	0.00	1
11	3	0	0.00	0.06	0.00	2	4	1	0.15	0.06	2.74	1	5	1	0.15	0.07	2.06	1
12	2	0	0.00	0.05	0.00	3	5	1	0.15	0.07	2.06	1	7	1	0.15	0.11	1.37	1
13	2	1	0.08	0.02	3.62	2	6	1	0.15	0.09	1.65	1	5	1	0.15	0.07	2.06	1
14	4	2	0.15	0.04	3.62	2	4	1	0.15	0.06	2.74	1	6	2	0.31	0.07	4.12	1
15	1	0	0.00	0.02	0.00	1	3	0	0.00	0.06	0.00	1	6	1	0.15	0.09	1.65	1
16	5	2	0.15	0.06	2.41	2	4	1	0.15	0.06	2.74	1	8	1	0.15	0.13	1.18	1
17	4	1	0.05	0.07	0.69	3	8	1	0.15	0.13	1.18	1	4	1	0.15	0.06	2.74	1
18	1	0	0.00	0.02	0.00	2	6	1	0.15	0.09	1.65	1	4	1	0.15	0.06	2.74	1
19	4	2	0.15	0.04	3.62	2	7	1	0.15	0.11	1.37	1	4	1	0.15	0.06	2.74	1
20	5	1	0.08	0.09	0.90	2	4	0	0.00	0.07	0.00	1	7	1	0.15	0.11	1.37	1
	74		0.09		1.81	2.1	91		0.09		1.28	1.05	101		0.15		2.22	1
					1.72						1.22						1.16	

Rat

35A	TotalMark	Matched	Frequenc	Frequency	Ratio	Light	TotalMark	Matched M	Frequenc	Frequenc	Ratio	Light	TotalMark	Matched	Frequenc	Frequency (	Ratio	Light
1	3	1	0.15	0.04	4.12	1	1	0	0.00	0.02	0.00	1	2	0	0.00	0.04	0.00	1
2	3	1	0.08	0.04	1.81	2	4	1	0.15	0.06	2.74	1	3	1	0.15	0.04	4.12	1
3	6	4	0.31	0.04	7.23	2	5	2	0.31	0.06	5.49	1	6	3	0.46	0.06	8.23	1
4	2	0	0.00	0.04	0.00	2	6	1	0.15	0.09	1.65	1	8	2	0.31	0.11	2.74	1
5	7	1	0.08	0.13	0.60	2	2	0	0.00	0.04	0.00	2	9	2	0.31	0.13	2.35	1
6	4	2	0.15	0.04	3.62	2	4	1	0.15	0.06	2.74	1	9	1	0.15	0.15	1.03	1
7	7	2	0.31	0.09	3.29	1	2	1	0.15	0.02	8.23	1	9	1	0.15	0.15	1.03	1
8	4	3	0.12	0.03	3.92	4	5	1	0.15	0.07	2.06	1	4	2	0.31	0.04	8.23	1
9	6	2	0.15	0.09	1.81	2	4	2	0.31	0.04	8.23	1	4	1	0.15	0.06	2.74	1
10	3	1	0.15	0.04	4.12	1	4	2	0.31	0.04	8.23	1	7	2	0.31	0.09	3.29	1
11	5	0	0.00	0.09	0.00	1	4	1	0.15	0.06	2.74	1	7	1	0.15	0.11	1.37	1
12	6	2	0.15	0.09	1.81	2	8	3	0.46	0.09	4.94	1	9	5	0.77	0.07	10.29	1
13	7	5	0.26	0.05	5.19	3	5	0	0.00	0.09	0.00	1	9	1	0.15	0.15	1.03	1
14	3	1	0.08	0.04	1.81	2	6	2	0.31	0.07	4.12	1	5	1	0.15	0.07	2.06	1
15	5	1	0.08	0.09	0.90	2	11	2	0.31	0.17	1.83	1	3	1	0.15	0.04	4.12	1
16	3	1	0.15	0.04	4.12	1	7	0	0.00	0.13	0.00	1	7	2	0.31	0.09	3.29	1
17	5	1	0.08	0.09	0.90	2	8	0	0.00	0.15	0.00	1	8	1	0.15	0.13	1.18	1
18	5	2	0.10	0.07	1.38	3	8	3	0.46	0.09	4.94	1	11	1	0.15	0.19	0.82	1
19	1	0	0.00	0.02	0.00	3	3	0	0.00	0.06	0.00	1	2	2	0.31	0.00	11.00	1
20	7	4	0.31	0.06	4.82	2	6	0	0.00	0.11	0.00	1	9	2	0.31	0.13	2.35	1
	85		0.14		2.57	2	102		0.17		2.66	1.05	131		0.25		3.56	1
																	3.17	

Matlab code output of One Wall Forced Choice data for rat 35. Highlighted cell indicates replaced value to generate 'true' ratio in analyses.

36B																			
	1	4	1	0.15	0.06	2.74	1	6	0	0.00	0.11	0.00	1	7	1	0.15	0.11	1.37	1
	2	4	1	0.15	0.06	2.74	1	2	1	0.15	0.02	8.23	1	6	1	0.15	0.09	1.65	1
	3	1	0	0.00	0.02	0.00	1	3	0	0.00	0.06	0.00	1	5	1	0.15	0.07	2.06	1
	4	5	2	0.31	0.06	5.49	1	4	0	0.00	0.07	0.00	1	8	1	0.15	0.13	1.18	1
	5	5	1	0.15	0.07	2.06	1	2	1	0.15	0.02	8.23	1	5	1	0.15	0.07	2.06	1
	6	3	1	0.15	0.04	4.12	1	4	1	0.15	0.06	2.74	1	3	1	0.15	0.04	4.12	1
	7	3	1	0.15	0.04	4.12	1	4	0	0.00	0.07	0.00	1	1	0	0.00	0.02	0.00	1
	8	4	1	0.15	0.06	2.74	1	3	0	0.00	0.06	0.00	1	2	1	0.15	0.02	8.23	1
	9	8	1	0.15	0.13	1.18	1	4	1	0.15	0.06	2.74	1	3	1	0.15	0.04	4.12	1
	10	6	1	0.15	0.09	1.65	1	3	1	0.15	0.04	4.12	1	4	1	0.15	0.06	2.74	1
	11	5	1	0.15	0.07	2.06	1	2	1	0.15	0.02	8.23	1	6	1	0.15	0.09	1.65	1
	12	7	1	0.08	0.13	0.60	2	5	1	0.15	0.07	2.06	1	5	1	0.15	0.07	2.06	1
	13	5	1	0.15	0.07	2.06	1	3	1	0.15	0.04	4.12	1	6	1	0.15	0.09	1.65	1
	14	4	1	0.15	0.06	2.74	1	3	0	0.00	0.06	0.00	1	5	3	0.46	0.04	12.35	1
	15	5	1	0.15	0.07	2.06	1	4	1	0.08	0.06	1.21	2	5	0	0.00	0.09	0.00	1
	16	4	1	0.15	0.06	2.74	1	4	0	0.00	0.07	0.00	1	7	1	0.15	0.11	1.37	1
	17	5	0	0.00	0.09	0.00	1	4	1	0.15	0.06	2.74	1	7	1	0.15	0.11	1.37	1
	18	7	1	0.15	0.11	1.37	1	6	0	0.00	0.11	0.00	1	4	1	0.15	0.06	2.74	1
	19	4	0	0.00	0.07	0.00	1	2	1	0.15	0.02	8.23	1	6	0	0.00	0.11	0.00	1
	20	4	1	0.15	0.06	2.74	1	5	1	0.15	0.07	2.06	1	4	0	0.00	0.07	0.00	1
		93		0.13		2.16	1.05	73		0.09		2.74	1.05	99		0.14		2.53	1
						1.40						3.06						2.90	

Rat																			
36A		TotalMark	Matched	Frequenc	Frequency	Ratio	Light	TotalMark	Matched	Frequenc	Frequenc	Ratio	Light	TotalMark	Matched	Frequenc	Frequency (	Ratio	Light
	1	4	0	0.00	0.07	0.00	1	2	0	0.00	0.04	0.00	1	4	1	0.15	0.06	2.74	1
	2	4	1	0.15	0.06	2.74	1	3	2	0.31	0.02	16.46	1	4	2	0.31	0.04	8.23	1
	3	2	1	0.15	0.02	8.23	1	4	0	0.00	0.07	0.00	1	1	0	0.00	0.02	0.00	1
	4	5	0	0.00	0.09	0.00	1	6	1	0.15	0.09	1.65	1	3	1	0.15	0.04	4.12	1
	5	2	0	0.00	0.04	0.00	1	3	0	0.00	0.06	0.00	1	4	2	0.31	0.04	8.23	1
	6	2	0	0.00	0.04	0.00	1	5	1	0.15	0.07	2.06	1	3	2	0.31	0.02	16.46	1
	7	2	2	0.31	0.00	9.00	1	1	0	0.00	0.02	0.00	1	3	1	0.15	0.04	4.12	1
	8	3	0	0.00	0.06	0.00	1	5	1	0.15	0.07	2.06	1	1	1	0.15	0.00	18.00	1
	9	3	1	0.15	0.04	4.12	1	3	0	0.00	0.06	0.00	1	1	1	0.15	0.00	18.00	1
	10	4	0	0.00	0.07	0.00	1	3	0	0.00	0.06	0.00	1	2	1	0.15	0.02	8.23	1
	11	2	1	0.15	0.02	8.23	1	5	0	0.00	0.09	0.00	1	1	0	0.00	0.02	0.00	1
	12	0	0	0.00	0.00	0.00	1	2	1	0.15	0.02	8.23	1	2	1	0.15	0.02	8.23	1
	13	8	0	0.00	0.15	0.00	1	4	1	0.15	0.06	2.74	1	2	2	0.31	0.00	18.00	1
	14	2	0	0.00	0.04	0.00	1	2	1	0.15	0.02	8.23	1	2	1	0.15	0.02	8.23	1
	15	3	1	0.15	0.04	4.12	1	5	0	0.00	0.09	0.00	1	1	0	0.00	0.02	0.00	1
	16	1	0	0.00	0.02	0.00	1	4	2	0.31	0.04	8.23	1	2	1	0.15	0.02	8.23	1
	17	3	1	0.15	0.04	4.12	1	2	0	0.00	0.04	0.00	1	1	1	0.15	0.00	18.00	1
	18	4	2	0.31	0.04	8.23	1	5	1	0.15	0.07	2.06	1	4	1	0.15	0.06	2.74	1
	19	4	0	0.00	0.07	0.00	1	4	2	0.31	0.04	8.23	1	4	0	0.00	0.07	0.00	1
	20	3	0	0.00	0.06	0.00	1	2	0	0.00	0.04	0.00	1	3	1	0.15	0.04	4.12	1
		61		0.08		2.44	1	70		0.10		3.00	1	48		0.15		7.78	1
						3.34						4.39						6.43	

Matlab code output of One Wall Forced Choice data for rat 36. Highlighted cell indicates replaced value to generate 'true' ratio in analyses.

```
%Name: Patricia Bacala
%Date: 12/18/2013
%File: 'Analyze.m'

clear all; clc;
num=xlsread('test.xlsx');

%for the cells in the excel file
for i=(2:2401)
    count(i)=0;
    matchedcount(i)=0;

if num(i,2)-num(i-1,2)>0.1
    count(i)=count(i)+1;
end
newcount=count';

%if the absolute difference between a cell and the next is greater than
0.09 (lever
%press) count increases to determine number of presses

if num(i,2)-num(i-1,2)>0.1 && num(i,3)>0
    matchedcount(i)= matchedcount(i) + 1;

end
newmatch=matchedcount';
end

%if the difference is greater than 0.09 and the value in the 3rd column is
%greater than 1 count as a matched lever press

for p=2:2401 %light counter
    lightcount(p)=0;
    if num(p,3)-num(p-1,3)>1
        lightcount(p)= lightcount(p) + 1;
    end
end

%creating array for lever presses
min1=sum(newcount(1:121));
min=[min1];

%creating array for matched presses
matchsum1=sum(newmatch(1:121));
matchsum=[matchsum1];

%adding to arrays by minute
for j=2:20
    min(j,:) = sum(newcount((121+120*(j-2)):(121+120*(j-1))));


for k=2:20
    matchsum(k,:) = sum(newmatch((121+120*(k-2)):(121+120*(k-1))));
```

```
end
end

%creating array for frequency of on or off matched
freqon1= matchsum(1,:)/min(1,:);
freqon=[freqon1];

for l=2:20
        freqon(l,:) = matchsum(l,:)/min(l,:);
end

freqoff1=1-freqon1;
freqoff=[freqoff1];

for m=2:20
    freqoff(m,:)= 1-freqon(m,:);
end
%
% %creating array and counter for the ratio of on to off
ratio1= freqon(1,:)/freqoff(1,:);
ratio=[ratio1];

for n=2:20
    ratio(n,:) = freqon(n,:)/freqoff(n,:);
end;
%
%
% %light counter
newlight=lightcount';
light1=sum(newlight(1:121));
light=[light1];

for q=2:20
    light(q,:) = sum(newlight((121+120*(q-2)):(121+120*(q-1))));
end

%excel header stuff
time=(1:20)';

%M=[time, min];
M=[time, min, matchsum, freqon, freqoff, ratio, light];

col_header={'Minute','Total Marks','Matched Marks','Frequency On',
'Frequency Off', 'Ratio', 'Light'};
xlswrite('test.xlsx',col_header,'Sheet2','A1:G1');

%write to excel file in second sheet
xlswrite('test.xlsx', M, 'Sheet2', 'A2:G21');
```

2/4/2014 10:31:44 AM

```
Welcome to Minitab, press F1 for help.
```

To generate the worksheet columns C1-C3, I copied the "Ratio" data from the tab "Light". To create the missing data,
I examined the maximum ratio for that animal, and rounded up (e.g. a ratio of 11.7 was the largest for animal 36, so I rounded to 12, and inserted it in the #DIV/0! cell, thereby generating a "true" ratio).

Refer to this file: http://it.minitab.com/en-us/support/documentation/Answers/Repeated_Measures_Design.pdf

The first question we can ask is are the means of the trials the same...
mean(1) = mean(2) = mean(3) = ... = mean(8) - basically, are the animals learning.
Your data are limited in that you only have two animals - not good for generating a mean.
The GLM procedure does the correct accounting, and the data MUST be in the form showed in the worksheet.

The repeated measures analysis also allows you to determine if the animals differ in their responses (that's the "Subject part). The outcome of this analysis is below.

General Linear Model: Ratio versus Subject, Trial (Light)

```
Factor          Type    Levels  Values
Subject         random       2  35, 36
Trial (Light)   fixed        8  1, 2, 3, 4, 5, 6, 7, 8


Analysis of Variance for Ratio, using Adjusted SS for Tests

Source          DF  Seq SS  Adj SS  Adj MS     F      P
Subject          1  1.1636  1.1636  1.1636  6.59  0.037
Trial (Light)    7  2.6975  2.6975  0.3854  2.18  0.162
Error            7  1.2357  1.2357  0.1765
Total           15  5.0968


S = 0.420147   R-Sq = 75.76%   R-Sq(adj) = 48.05%


Unusual Observations for Ratio

Obs    Ratio      Fit   SE Fit  Residual  St Resid
  4  1.74519  2.31299  0.31511  -0.56780     -2.04 R
 12  3.42014  2.85234  0.31511   0.56780      2.04 R

R denotes an observation with a large standardized residual.
```

My interpretation is that the animals do not develop a greater preference for the light on condition, since the mean ratio is the same for each trial. A subsequent interpretation is that the animals differ in their light preference. Reviewing the data, animal 36 has a larger preference than animal 35.
This outcome can be confirmed more simply using a t-test. For the example below, I use a two-tailed test (just the baseline). The statistical outcome is different because of the accounting for the variation that is better handled in the GLM procedure. You get the point,

Two-Sample T-Test and CI: Ratio, Subject

```
Two-sample T for Ratio
```

```
Subject  N   Mean  StDev  SE Mean
35       8  1.577  0.283     0.10
36       8  2.116  0.694     0.25


Difference = mu (35) - mu (36)
Estimate for difference:  -0.539
95% CI for difference:  (-1.139, 0.060)
T-Test of difference = 0 (vs not =): T-Value = -2.04  P-Value = 0.072  DF = 9
```

We asked whether rats distinguished between on and off. To examine this question, you have to set the baseline expectation to 1. So for a t-test I just used the one-sample option and entered the summarized data. Top row below is 35, bottom row is 36.

```
Test of mu = 1 vs not = 1
N    Mean    StDev  SE Mean       95% CI          T      P
8  1.5768  0.2825   0.0999  (1.3406, 1.8130)  5.77  0.001
8  2.116   0.694    0.245   (1.536, 2.697)    4.55  0.003
```

This stuff should get you going. Note that for this file I ONLY used the data from the "Light" tab. You can add your "Left/Right" and "One Wall" data to this file in separate columns and then just choose which column to analyze. * is the empty cell indicator (e.g. if you don't have a separate Trial column for each experiment, just enter * in the spots with no trials). Give it a try and see what happens.

2/4/2014 6:12:14 PM

This test is just a variation of the test perfromed by Dr. Ingram. I changed the alternative hypothesis so that it was greater than 1.
My interpretation is that the subjects can distinguish between on and off light. 35 is the top row, 36 is the bottom.

One-Sample T

```
Test of mu = 1 vs > 1


                              95% Lower
N    Mean   StDev  SE Mean      Bound     T      P
8  2.116  0.694     0.245      1.651  4.55  0.001
8  1.5768  0.2825   0.0999     1.3876  5.77  0.000
```

This test compared the baseline with the autoshaping (no light) data. The subjects performed equally on the tasks. The phase made a differences on the total number of lever presses and demonstrated change over the subsequent trials.

General Linear Model: Lever Press versus Subjects, Phase, Trial_auto

```
Factor       Type    Levels  Values
Subjects     random       2  35, 36
Phase        fixed        2  auto, base
Trial_auto   fixed        9  1, 2, 3, 4, 5, 6, 7, 8, 9


Analysis of Variance for Lever Press, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subjects	1	2268	2268	2268	0.98	0.335
Phase	1	81603	20098	20098	8.72	0.009
Trial_auto	8	90591	90591	11324	4.92	0.003
Error	17	39167	39167	2304		
Total	27	213629				

S = 47.9992 R-Sq = 81.67% R-Sq(adj) = 70.88%

This test confirms that the autoshaping data and baseline data are not equal, which is good and demonstrates that autoshaping was an effective method for training the rats to press the lever.

Two-Sample T-Test and CI: Lever Press, Phase

Two-sample T for Lever Press

Phase	N	Mean	StDev	SE Mean
auto	18	134.7	88.1	21
base	10	22.00	3.23	1.0

Difference = mu (auto) - mu (base)
Estimate for difference: 112.7
95% lower bound for difference: 76.5
T-Test of difference = 0 (vs >): T-Value = 5.42 P-Value = 0.000 DF = 17

I compared the light always on trials with the autoshaping no light trials to see if there was an effect. This test suggests that there is a difference, but I do another test next testing the same idea.

General Linear Model: Lever(A/ON) versus Subject(A/ON), Phase_ON, ...

Factor	Type	Levels	Values
Subject(A/ON)	random	2	35, 36
Phase_ON	fixed	2	auto, on
Trial_ON	fixed	9	1, 2, 3, 4, 5, 6, 7, 8, 9

Analysis of Variance for Lever(A/ON), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject(A/ON)	1	2328	2328	2328	0.90	0.359
Phase_ON	1	42001	101761	101761	39.23	0.000
Trial_ON	8	99593	99593	12449	4.80	0.004
Error	15	38911	38911	2594		
Total	25	182832				

S = 50.9320 R-Sq = 78.72% R-Sq(adj) = 64.53%

In this GLM I test the two conditions using only the last 4 trials of the autshaping (no light) with the always on trials.
I thought this was most representative of the subject behavior because learning had occured at this point, whereas it hadn't in early trials.

General Linear Model: Lever(A/ON) versus Subject(A/ON), Phase_ON, ...

```
Factor          Type    Levels  Values
Subject(A/ON)   random       2  35, 36
Phase_ON        fixed        2  auto, on
Trial_ON        fixed        4  1, 2, 3, 4


Analysis of Variance for Lever(A/ON), using Adjusted SS for Tests

Source              DF  Seq SS  Adj SS  Adj MS     F      P
Subject(A/ON)        1     870     870     870  0.41  0.541
Phase_ON             1    2601    2601    2601  1.24  0.303
Trial_ON             3   10810   10810    3603  1.71  0.251
Phase_ON*Trial_ON    3    2413    2413     804  0.38  0.769
Error                7   14742   14742    2106
Total               15   31436


S = 45.8908   R-Sq = 53.11%   R-Sq(adj) = 0.00%
```

This test just confirms that the two subjects performed equally.

Two-Sample T-Test and CI: Lever(A/ON), Subject(A/ON)

```
Two-sample T for Lever(A/ON)

                                  SE
Subject(A/ON)    N   Mean  StDev  Mean
35              13  152.0   82.4    23
36              13  170.9   90.8    25


Difference = mu (35) - mu (36)
Estimate for difference:  -18.9
95% CI for difference:  (-89.3, 51.4)
T-Test of difference = 0 (vs not =): T-Value = -0.56  P-Value = 0.583  DF = 23
```

This examines the effect of operant conditioning on the ratio of the frequency of on vs. frequency of off pressings. The missing values of ratios were substituted with the same method described above.
The ratios for subjects and trials were equal, respectively. Thus, my interpretation is that operant conditioning did not result in a preference for the light stimulus.

General Linear Model: Ratio_Op versus Subject_Op, Trial_Op

```
Factor      Type    Levels  Values
Subject_Op  random       2  35, 36
Trial_Op    fixed        5  1, 2, 3, 4, 5


Analysis of Variance for Ratio_Op, using Adjusted SS for Tests

Source      DF    Seq SS    Adj SS    Adj MS     F      P
Subject_Op   1  0.042988  0.042988  0.042988  7.44  0.053
Trial_Op     4  0.109959  0.109959  0.027490  4.76  0.080
Error        4  0.023113  0.023113  0.005778
Total        9  0.176059


S = 0.0760141   R-Sq = 86.87%   R-Sq(adj) = 70.46%
```

The one sample T test was used to determine if the subjects could distinguish between an on or off light stimulus.

Subject 35 could distinguish but rat 36 could not. A two sample T test and the GLM of the subjects support that the subjects have equal preference for the stimulus.

One-Sample T : Operant

```
Test of mu = 1 vs < 1

                                  95% Upper
(Subject)N       Mean    StDev  SE Mean      Bound      T      P
(35)5          1.0160   0.1690   0.0756     1.1771   0.21  0.579
(36)5          0.8847   0.0684   0.0306     0.9499  -3.77  0.010
```

The effect of subject, lever, and trial were tested on the ratio of the one wall (OW) trials. Because the means for these resspective values are
greater that 0.05, we can conclude that none of these variable had an effect. Therefore, the subjects demonstrated equal preference for the stimulus.
The subjects had equal prefence for the two levers, and the subjects did demostrate a change over subsequent trials in their preference. There was also
no apparent effect of the interaction between the factors (lever*trial and subject*lever).

General Linear Model: Ratio_OW versus Subject_OW, Lever_OW, Trial_OW

```
Factor       Type    Levels  Values
Subject_OW   random       2  35, 36
Lever_OW     fixed        2  A, B
Trial_OW     fixed        3  1, 2, 3


Analysis of Variance for Ratio_OW, using Adjusted SS for Tests

Source                DF  Seq SS  Adj SS  Adj MS      F      P
Subject_OW             1   3.116   3.116   3.116   6.42  0.239
Lever_OW               1   8.115   8.115   8.115  16.72  0.153
Trial_OW               2   3.223   3.223   1.612   0.93  0.466
Lever_OW*Trial_OW      2   5.201   5.201   2.600   1.50  0.326
Subject_OW*Lever_OW    1   0.485   0.485   0.485   0.28  0.625
Error                  4   6.927   6.927   1.732
Total                 11  27.068


S = 1.31592   R-Sq = 74.41%   R-Sq(adj) = 29.63%
```

Subjects both individually demonstrated the ability to discriminate the presence of the light stimulus.
35 is on top and 36 is on the bottom.

One-Sample T for One Wall

```
Test of mu = 1 vs > 1


                                 95% Lower
N   Mean   StDev  SE Mean         Bound     T      P
6  2.207  0.717    0.293         1.617  4.12  0.005
6  3.230  2.070    0.845         1.527  2.64  0.023
```

The effect of these factors in the L/R setup on the ratio were tested. Because the ratios were greater than alpha, none of these factors had an effect on the ratio.

General Linear Model: Ratio_LR versus Subject_LR, Lever_LR, Trial_LR

```
Factor       Type    Levels  Values
Subject_LR   random       2  35, 36
Lever_LR     fixed        2  L, R
Trial_LR     fixed        2  1, 2


Analysis of Variance for Ratio_LR, using Adjusted SS for Tests

Source                DF  Seq SS  Adj SS  Adj MS     F      P
Subject_LR             1  2.2371  2.2371  2.2371  1.94  0.396
Lever_LR               1  0.2066  0.2066  0.2066  0.18  0.745
Trial_LR               1  0.9399  0.9399  0.9399  1.99  0.253
Subject_LR*Lever_LR    1  1.1529  1.1529  1.1529  2.44  0.216
Error                  3  1.4192  1.4192  0.4731
Total                  7  5.9557


S = 0.687800   R-Sq = 76.17%   R-Sq(adj) = 44.40%
```

Subject 35 is on top, 36 on bottom. The subjects are able to discriminate between the the presence of the light stimulus.

One-Sample T for L/R

```
Test of mu = 1 vs > 1

                                 95% Lower
N   Mean   StDev  SE Mean         Bound     T      P
4  2.445  0.785    0.393         1.521  3.68  0.017
4  3.503  0.790    0.395         2.573  6.34  0.004
```

These data for the L/R setup were combined for total values for the trial and the effects were measured on the ratio. None of the factors were significant.

General Linear Model: Ratio_cmb versus Subject_cmb, Trial_cmb

```
Factor       Type    Levels  Values
Subject_cmb  random       2  35, 36
Trial_cmb    fixed        2  1, 2


Analysis of Variance for Ratio_cmb, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS   Adj MS     F      P
Subject_cmb              1  0.05437  0.05437  0.05437  0.11  0.798
Trial_cmb                1  0.00604  0.00604  0.00604  0.01  0.931
Subject_cmb*Trial_cmb    1  0.50480  0.50480  0.50480    **
Error                    0        *        *        *
Total                    3  0.56520

** Denominator of F-test is zero or undefined.


S = *
```

When the data were combined for the left and right lever trials, the subjects do not demonstrate the ability to discriminate the presence of the light stimulus.

One-Sample T : Ratio_cmb of each subject

Test of mu = 1 vs > 1

N	Mean	StDev	SE Mean	95% Lower Bound	T	P
2	1.431	0.667	0.472	-1.547	0.91	0.264
2	1.354	0.338	0.239	-0.155	1.48	0.189

General Linear Model: Ratio_COW versus Subject_COW, Trial_COW (combined one wall)

Factor	Type	Levels	Values
Subject_COW	random	2	35, 36
Trial_COW	random	3	1, 2, 3

Analysis of Variance for Ratio_COW, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Subject_COW	1	6.233	6.233	6.233	2.73	0.240
Trial_COW	2	6.446	6.446	3.223	1.41	0.414
Error	2	4.561	4.561	2.281		
Total	5	17.240				

S = 1.51018 R-Sq = 73.54% R-Sq(adj) = 33.86%