Student Investigator: Brady M. Salli **Project Title:** Parametric Photocues as a Synchronizer of Circadian Rhythms in an Arctic Hibernator.

Abstract and Specific Aims

Survival of an organism is largely contingent upon appropriate timing of the onset and cessation of many physiological and behavioral processes. Although the duration of these key, rhythmic processes are generated endogenously and show marginal intra-species variation, they often require and respond to external cues or zeitgebers. The reliance and utilization of photic zeitgebers such as the light/dark cycle of a 24h day are highly conserved phenomena observed in all domains of life. High latitude environments, however, bring about periods of continuous light during the summer, challenging the ability of polar animals to remain rhythmic; many organisms become arrhythmic during this period, deviating significantly from their normal patterns of behavior. A notable exception are summer active arctic ground squirrels (*Urocitellus parryii*), which display 24h rhythmicity in body temperatures (T_b) following emergence from their winter hibernacula. It remains unknown if arctic ground squirrels can synchronize or entrain circadian rhythms to parametric photic cues such as discrete changes in intensity and/or spectral quality of light. I propose to test the hypothesis that arctic ground squirrels can entrain circadian rhythms to parametric photic cues. I will test this hypothesis by addressing the two specific aims listed below.

Specific aim 1: Quantify and evaluate the free-running period of captive arctic ground squirrels.

Specific aim 2: Qualify and evaluate the ability of captive arctic ground squirrels to entrain T_b and loco-motor activity to parametric photic cues.

Introduction

Many of the behavioral and physiological patterns that animals display oscillate through a 24h period. These, so called circadian rhythms, oscillate through a near 24h period allowing organisms to anticipate various changes in the environment, such as the seasonal and diel changes in photoperiod. The degree to which circadian rhythms are synchronized or entrained is largely dependent upon the availability and exposure of the organism to external cues, or zeitgebers. In temperate latitudes, the light-dark (LD) cycle within a 24h period synchronizes diurnal rythmicity in many species (Aschoff, 1989).The observed spectral composition of light, dependent upon the rotation of the earth, is thought to be an effective astronomical zeitgeber that entrains endogenous daily rhythms (Krüll, Demmelmeyer&Remmert, 1985). In mammals, molecular clocks within cells of the hypothalamic suprachiasmatic nucleus (SCN) act as timekeepers, taking in photic input through the retina of the eyes (Menaker, Moreira et al., 1997). These light-cues are then transduced in the brain to elicit systemic changes in physiology and behavior.

Polar environments are characterized by periods of continuous light or dark during the summer and winter, respectively. It has been hypothesized that the extreme variation in annual photoperiod has led to many arctic residents abandoning diel rhythms (van Oort 2005). For example, animals such as caribou (*Rangifer tarandus)* and ptarmigan (*Lagopus mutus)* have been shown to become arrhythmic during conditions of constant light (Eloranta, Timisjaervi et al., 1995) whereas arctic ground squirrels

(*Urocitellus parryii*) and the arctic- migrant bird, Lapland longspur (*Calcarius lapponicus)*, are exceptions to the arrhythmic behavior seen in many arctic animals; both maintain rhythmicity despite the constant light of the polar summer. It remains unclear, however, if maintenance of diel rhymicity is a result of parametric photoentrainment or entrainment to non-photic zeitgebers such as ambient temperature.

During the hibernation season, arctic ground squirrels (AGS) remain burrowed in hibernacula where they alternated between long bouts of torpor interspersed with inter bout arousals to euthermic T_b. Currently, there is much debate concerning the function and persistence of circadian rhythms during steady state torpor. It has been reported, for example, that the expression of certain clock genes within the suprachiasmatic nucleus do not oscillate for several days of torpor in European hamsters, but are continuously expressed (Revel, et al 2007). This contrasts reports of oscillating gene expression during torpor in Djungarian hamsters (Herwig, et al 2006). In any case, there is no outward manifestation of circadian rhythms in T_b of AGS during hibernation; thus, the question remains as to what mechanisms accounts for the consolidation of behavior and physiology seen in summer active AGS following emergence from their winter burrows. Tb loggers affixed to free living AGS suggest that Tb of male AGS remain arrhythmic following the terminal arousal bout until emergence from hibernacula (Barnes, Buck, in review). This suggest that rhymicity in T_b requires external zeitgebers such as photo cues. Because sunlight is continuous throughout the active season, it is probable that parametric photocues such as the oscillation in light intensity and color temperature throughout the polar day are responsible for entrainment; primary, non-parametric photocues such as dawn and dusk are absent around the winter and summer solstices in polar regions.

The persistent circadian rhythms observed in AGS at high latitudes remain to be fully understood. The current research aims to advance arctic systems science by examining T_b and locomotor patterns of an arctic hibernator and the possible external cues guiding adaptations. Moreover, a more sophisticated understanding of how an organism adapted to the extreme Arctic regulates circadian rhythms increases the knowledge of rhythms in general and can be applied to other scientific disciplines such as biomedicine. Errors in circadian cycling, for instance, have been implicated in psychological disorders such as depression and schizophrenia (Verma, Hellemans et al., 2010). Additionally, disturbances of rest/active cycles have been correlated with an increased risk for cardiac diseases in the elderly (Paudel, et al 2011).

Experimental Design

The aims of this study are to 1) establish the free running periodicity of T_b and locomotor activity in captive AGS and 2) evaluate the capability of AGS to entrain T_b and locomotor rhythms to parametric photocues. Together, these data will be used to test the ***hypothesis that arctic ground squirrels can synchronize or entrain circadian rhythms to parametric photic cues such as changes in intensity and/or spectral quality of light.*** The protocols for testing this hypothesis are given below:

Experimental objectives:

- Implant DSI temperature transmitters into captive AGS
- Monitor T_b and locomotor activity of captive AGS in constant dark and constant light.
- Design and replicate the varying parametric photocues present during the polar summer.

Objective 1: Implant DSI temperature transmitters into captive AGS.

In order to monitor T_b, AGS will be outfitted with temperature transmitters (Data Systems International) inserted into the peritoneal cavity. DSI transmitters report T_b in real-time allowing preliminary analysis to be performed immediately.

Methodology

Surgeries will be performed on ten captive AGS in the vivarium of the Conoco Phillips Integrated Science Building, University of Alaska Anchorage. All surgical protocols will comply with IACUC guidelines and regulations.

Surgical preparation: DSI transmitters (~10 g) will be cleaned with Terg-A-zyme (Alconox, Inc.) prior to chemical sterilization. Transmitters will then be chemically sterilized using Cidex activated dialdehyde solution (Johnson & Johnson Co). All surgical instruments will be wrapped in drape and autoclaved no more the one week before surgeries. Subjects will fast 2-4 hours before surgery and receive 2mg/kg of Ketoprofen. Induction of 0.5-5% Isoflurane will be administered in an anesthetic chamber. Inducted squirrels will then be maintained using a non-rebreath system attached to a face mask throughout the duration of the surgery (approximately 30 minutes). The incision site will be draped with sterile draping material. A glass bead sterilizer will be used to re-sterilize instruments between surgeries.

Surgical procedure: Inducted squirrels will be placed dorsally on a heated water pad. The abdomen will be shaved with clippers along the incision site which will then be scrubbed 3 times with betadine-soaked gauze. The first two scrubs will be rinsed with clean water and the third scrub with 70% alcohol. A 2cm midline incision will be made through the skin using a #15 scalpel. The subcutis and fat will be bluntly dissected using mosquito forceps to expose the linea alba. The linea alba will be picked up using fine rat tooth forceps and a stab incision will be made using a #15 scalpel. The incision is extended along the linea alba spreading apart the fasial plane. The transmitter is then inserted into the peritoneal cavity and covered with fat. The linea alba will then be closed using monofilament absorbable sutures. Alternate sutures for the abdomen and skin will be accompanied by the application of a thin bead of surgical glue using a 25g needle.

Subjects will be monitored closely for 3 hours after the surgery and surgery sites will be checked 1,2,3,5,7,9 and 14 days after the surgery. Antibiotics will be given at the dose and frequency prescribed by veterinary services.

Objective 2: Monitor T_b and locomotor activity of captive AGS in constant dark and constant light.

A necessary component of any circadian rhythms study is to quantify the periodicity of free-running activity. Because no endogenous rhythm is exactly 24h, animals held in a static environment (no zeitgebers) will delay or advance the onset of activity by some time increment each day. For example, a animal that has a free-running period of 23h and arouses each morning at 6:00 am will arouse 1h earlier each day it is held in static conditions (5:00 am the first day, 4:00 am the second day, etc.). Data collected from these experiments will be used to determine the lighting schedules described in Objective 3.

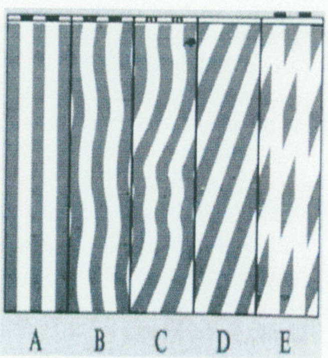
Methodology

Ten AGS, housed in environmentally controlled chambers and outfitted with DSI temperature transmitters, will be placed in individual cages outfitted with infra- red sensors to detect locomotor activity. Food and water will be provided *ad libitum.* AGS will first be exposed to 20h of light (intensity = 1200 lux) and 4h of darkness (0.1 lux) (20L:4D) for 10 days or until all animals show entrainment. Once entrainment has been demonstrated, AGS will be placed in constant darkness (DD) for 5 days. Animals will then be placed back on 20L:4D for 10 days, followed by 5 days of constant light (LL).

Monitor T_b: A DSI receiver placed beneath each cage will transmit core body temperature data to a computer every 5 minutes during the experiment. The associated software allows for real-time tracking and plotting of T_b versus time; however, ClockLab software will be used to analyze both T_b and locomotor activity.

Monitor locomotor activity: A shift in circadian activity will indicate a behavioral (locomotor activity) or physiological (T_b) change that corresponds to synchronization to a zeitgeber. ClockLab software will generate a history of the behavior throughout the experiment. **Figure 1** depicts a conceptual schematic of possible outcomes of behavior patterns.

Figure 1



Possible correspondence between a behavior and a zeitgeber. Top=zeitgeber. adopted from (Krüll, 1985)

The adjacent figure illustrates a relationship between a zeitgeber and the entrained behavior. A and B show synchronization with B having less distinct correspondence than A. C represents an even weaker zeitgeber with some of the behavior "free running."D has no zeitgeber; behavior is free running without any known external stimuli. E, an external cue deteriorates free-running (Krüll,Demmelmeyer, & Remmert, 1985). Actograms for animals under DD or LL should correspond to the patterns of behavior similar to **Figure 1**, panel D. Though behavioral profiles will be evaluated after the experiment, the software allows for real-time analysis of locomotor activity.

Anticipated outcomes

Early investigations focusing on circadian rhythms of arctic rodents report a wide range of values in circadian periods under LL and DD conditions (Swade & Pittendrigh, 1967). Limited data is available for AGS as most of these studies were multi-species surveys. Additionally, the methods for measuring activity and light intensity reflect the technology available at that time. A general rule, however, is that diurnal animals will shorten their active period under LL condition and the converse true for DD. I anticipate this observation will also be seen in this study. That is, AGS under LL will initiate the onset of activity and corresponding T_b later each day and AGS under DD will initiate the onset earlier, generating an actogram similar to that of **Figure 1**, panel D.

Objective 3: Design and replicate the varying parametric photocues present during the polar summer.

Data acquired from the Barrow Environmental Observatory have been used to establish minimum and maximum light intensities and color temperatures. These parameters will be used as boundaries during light and color temperature manipulations. Environmental conditions, data acquisition and animal care will be the same as described under Objective 2.

Methodology

Ten AGS, housed in environmentally controlled chambers and outfitted with DSI temperature transmitters, will be placed in individual cages outfitted with infra- red sensors to detect locomotor activity. Food and water will be provided *ad libitum.* AGS will first be exposed to 20h of light (intensity = 1200 lux) and 4h of darkness (0.1 lux) (20L:4D) for 10 days or until all animals show entrainment. Once entrainment has been demonstrated, AGS will be placed in DD for 5 days. The switch from DD to the following light experiments will be determined from data collected during the LL and DD experiments described under Objective 2. The duration for each experiment is 15 days followed by a 10 day break to entrain animals back to (20L:4D).

Light Intensity Experiment: 20h of bright light (1200 lux): 4h of low light (300 lux) with a constant color temperature (6500 K).

Color Temperature Experiment: 20h of white light (6500 K): 4h of red light (3500 K) with a constant light intensity (1300 lux).

Intensity/Color Experiment: 20h of bright white light (1200 lux): 4 hours of low red light (300 lux).

Anticipated outcomes

If parametric photocues are the primary zeitgeber for summer active AGS, I anticipate that at least one of the experimental protocols listed above will entrain animals. Possible explanations for a failure to entrain to these parameters are **1)** AGS do not use parametric photocues as a primary zeitgeber and **2)** The parametric photocues tested are not the photocues observed by free living AGS.

Budget Outline

Below is an itemized list of services and supplies along with the requested amount of funding. Justification for these items is given under the Budget Justification section.

Category	**AHIF**
Travel	
Domestic	
RT to San Francisco, CA	$800
Lodging for 5 days	$500
Per diem for 5 days	$300
Total domestic Travel	$1600
Total Travel	**$1,600**
Services	
Vivarium suite rental	$390
Vivarium tech support	$608
Veterinary services	$360
TOTAL SERVICES	**$1,358**
Supplies	
Food	$400
Nesting Material	$400
Rodent cages	$600
Misc. lab supplies (gloves, needles, etc.)	$200
Poster	$200
TOTAL SUPPLIES	**$1,800**
Total Funding Requested	**$ 4,758**

Budget Justification

Travel: This money will allow me to present my results at a national conference. The Society for Integrative and Comparative Biology hosts an annual meeting for scientists to present and discuss projects. Scheduled dates and location for the 2013 conference are Jan 3-7 in San Francisco, CA.

Vivarium suite rental: AGS will be housed within the UAA Vivarium. Rental space for this project is billed at $78.16/month. The total amount reflects 4 months of rental charges rounded to the nearest dollar.

Vivarium tech support: This money will cover basic amenities including assistance with husbandry and monitoring the overall welfare of animals.

Veterinary services: This money will enable me to receive surgical training and supervision from an IACUC approved veterinarian. The charges reflect 5 hours of services at $90/hour. 2 of the 5 hours are included to cover costs in the event there are post-surgery complications that require veterinary expertise.

Food and Nesting material: This money will be used to feed and supply the animals for the duration of the experiment.

Rodent cages: Existing cages within the Vivarium do not allow mounting of infra-red sensors. This money will allow me to purchase cages with meshing on all four sides (current cages only have meshing on the front and bottom).

Miscellaneous lab supplies: This money will cover the cost of day to day supplies such as gloves and the use of solutions and surgical instruments.

Poster: This money will be used to print and laminate a poster of my results to be presented at the national conference for the Society for Comparative and Integrative Biology.

References

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Project Timeline

The anticipated start date for this project is no later than August 15th, 2012. The target end date is no later than December 15th, 2012. The table below gives an overview of important tasks and the times they will be completed by.

Task	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Conduct Surgeries	■								
Conduct LL and DD free running experiments		■							
Data analysis		■	■						
Conduct parametic lighting experiments		■	■	■					
Data analysis				■	■				
Present results at national conference			■			■			
Write-up for publication / submit report to AHIF							■	■	
Presentation of results at UAA Undergraduate Research Forum									■