PATTERNS OF WHEEL RUNNING IN BULBECTOMIZED MICE

By

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Thesis

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This Thesis by *Anna Rinko* requirement for Honors in Biology.

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is accepted in its present form as satisfying the thesis

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Biography

Anna Rinko was born in Fredericksburg, VA, and grew up in King George County. She graduated Valedictorian from King George High School concurrently with her Associates of Arts and Sciences degree from Rappahanock Community College. She will graduate from the University of Mary Washington with a Bachelor of Science, having double majored in Biology and Communication & Digital Studies and minoring in Neuroscience. She is a member of the Phi Beta Kappa, Chi Beta Phi, and Lambda Pi Eta honor societies.

Acknowledgements

I would like to thank Dr. Waters for all of his support, guidance, advice, and Excel expertise. Nicole Taylor greatly assisted with mouse care and testing, and her expert pipetting skills were invaluable. I would like to thank Dr. O'Dell, Dr. Dolby, and Dr. Stahlman for their guidance as my committee members, and Dr. Stahlman for graciously providing us with lab space this year. I would also like to thank Dr. Cappola of Randolph Macon College for conducting and providing the site for olfactory bulbectomies.

Abstract

This work aims to better characterize the hyperlocomotor phenotype of olfactory bulbectomized (OBX) mice, a premiere model of depression. Characteristics of OBX mice mimic symptoms of human depression and include anhedonia, anxiety, hyperactivity, and hormonal changes. We previously examined anhedonia in this model by providing OBX mice with a reinforcing stimulus in the form of a running wheel, anticipating that these mice would have decreased wheel interaction due to their documented attenuated interest in rewards. Contrary to our expectations, the OBX mice ran significantly more than controls. We concluded that this resulted from the hyperactivity phenotype that is present in OBX mice. To differentiate whether this behavior was caused by hyperactivity or a true absence of anhedonia, we utilized a sorter system to limit access to an externally housed running wheel for a period of three weeks. This design forced mice to undergo a 30 second wait period to gain wheel access, allowing us to better determine motivated vs stereotypic behavior. These behavioral data were complimented by an evaluation of social dominance in dyads of mice and corticosterone levels. We found that OBX mice spent significantly less time interacting with the running wheel, showed disruptions to their circadian pattern of activity, and had higher fecal corticosterone levels at day 21 of the experiment. However, we observed no differences between running speed or entries into the wheel cage. In fact, cage entries actually increased slightly ($p = 0.06$) among OBX mice. We interpret these changes in wheel engagement as anhedonia, while the increased cage entries reflect hyperactivity in OBX mice. These findings highlight the anhedonic and hyperactive phenotypes of this model, and apply these to wheel running behavior. We also provide supporting evidence for circadian disruptions and the presence of higher corticosterone in OBX mice.

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Introduction

Human depression is a devastating condition and is therefore an active subject of research. A premiere mouse model for this condition is olfactory bulbectomized (OBX) mice, in which the olfactory bulb is surgically removed. These mice show behavioral and physiological changes that resemble the symptoms of human depression, including hyperactivity, impaired HPA axis function (reviewed in Harkin et al. 2003), aberrant social behavior (Richardson and Scudder 1970), and decreased interest in rewards (anhedonia) (Almeida et al. 2017).

Researchers have speculated that these symptoms are the result of changes to the rodent limbic system, of which the olfactory bulbs are an important input. Other evidence that bulbectomy's effects extend to non-olfactory brain regions include OBX rat studies which have demonstrated degeneration of neurons in areas such as the amygdala, hippocampus, and cortex. Of particular interest is the fact that anosmia resulting from other methods such as artificially damaging the nasal cavity does not lead to the behavioral changes documented in this model. These findings indicate that the symptoms present in the OBX model are likely the result of anatomical and physiological changes to the limbic system rather than mere anosmia (reviewed in Song 2005). This link between bulbectomy and changes in the hippocampus has been confirmed in a study examining the time-course of physiological and behavioral changes in OBX mice (Almeida et al. 2017). Furthermore, some studies suggest that higher corticosterone concentrations are also present in this model, which may be indicative of HPA axis changes. However, these stress hormone changes are disputed (reviewed in Harkin et al. 2003).

These changes that occur in OBX mice provide an important means by which to study depression and its treatments. The behavioral symptoms displayed by these mice are reduced when they are given antidepressants. This model has thus been used to test and develop new pharmaceutical therapies for depression (Morales-Medina 2017). Depression is a difficult condition to mimic in research, but OBX rodents are a promising animal model. Better characterization of the behavioral and hormonal effects of bulbectomy could thus potentially advance the treatment of human depression.

We were particularly interested in exploring the nature of these behavioral changes in a more ecologically valid social setting. Human depression can have severe social consequences; therefore, putting OBX mice in a social context rather than isolating them may more accurately mimic the human condition and subsequent social costs for the depressed individual. Using social colonies, we intended to perform a deep investigation of the anhedonic and hyperactive behavioral changes that are reported in OBX mice.

Wheel running behavior is an ideal variable that can be used to explore anhedonic effects in this model. Mice find a running wheel to be an intrinsically reinforcing stimulus, and they compete for its use (Belke and Pierce 2014). Providing OBX mice with a running wheel allows the dual examination of both reward seeking behavior and changes to their social status.

We previously performed research in which we provided OBX colonies (of 3 mice) with free access to a running wheel in their home cage, expecting these mice to run less due to their anhedonia. Surprisingly, the OBX mice ran significantly more than their control counterparts throughout the course of the experiment (Fig. 10). However, we could not

determine whether this difference was indicative of an absence of anhedonia or simply the result of stereotypic hyperactivity that is also associated with this model.

Thus, in the current experiment, we placed the running wheel located in an accessory cage, and access to this cage was dependent on *completing an operant task* by successfully navigating a series of RFID (radio-frequency identification) gated doors. This design allows us to discern whether OBX wheel running is a motivated or stereotypic behavior. The setup also limited wheel access to one mouse at any given time, allowing us to easily attribute wheel revolutions to individual mice for more fine-grained analyses (something that we were unable to accomplish in our previous research). These OBX colonies were contrasted with control colonies. Thus the primary hypotheses we tested include:

Hypothesis 1: The OBX procedure decreases motivation to run on a wheel (*anhedonia*)

Hypothesis 2: Social rank influences motivation to run on a wheel, and this relationship is disrupted by the OBX procedure (*aberrant social behavior*)

Hypothesis 3: Higher circulating corticosterone concentrations are present OBX mice. (*dysfunctional regulation of circulating corticosterone*)

Materials and Methods

Standard type 3 cages (425 x 266 x 185 mm) served as home cages and were connected to smaller accessory wheel cages (160 x 207 x140 mm) by a sorter system (Figs. 1 and 2; Phenosys, Berlin, Germany). This system utilizes RFID readers which recognize RFID chipped mice that approach a series of two gates between the two cages. Initial recognition triggers the first gate to close, preventing other mice from entering. The mouse must then wait thirty seconds before the second gate opens, providing the mouse with wheel cage access. The first gate immediately opens when it detects the mouse approaching the

sorter system to vacate the accessory wheel cage. Six test mice were used to set up and calibrate the system correctly before experimental mice were given access.

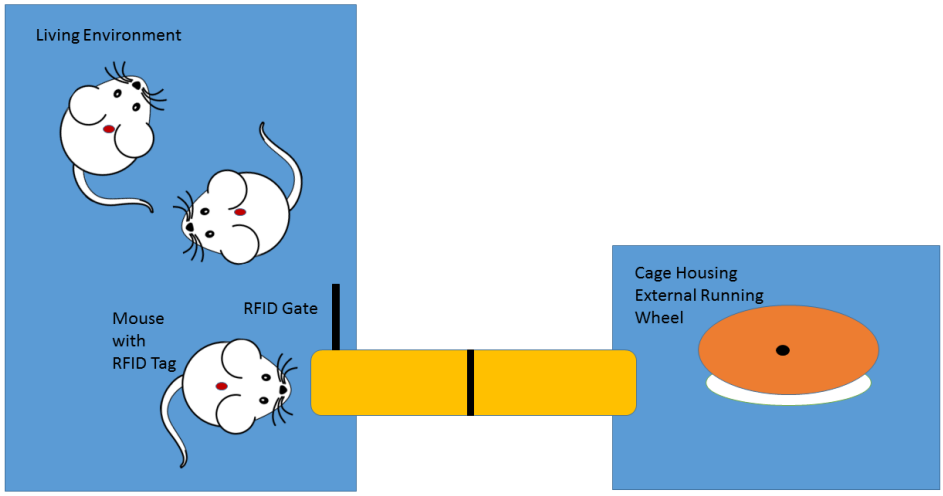

Fig 1: Cage layout. This diagram illustrates the experimental design used in this experiment.

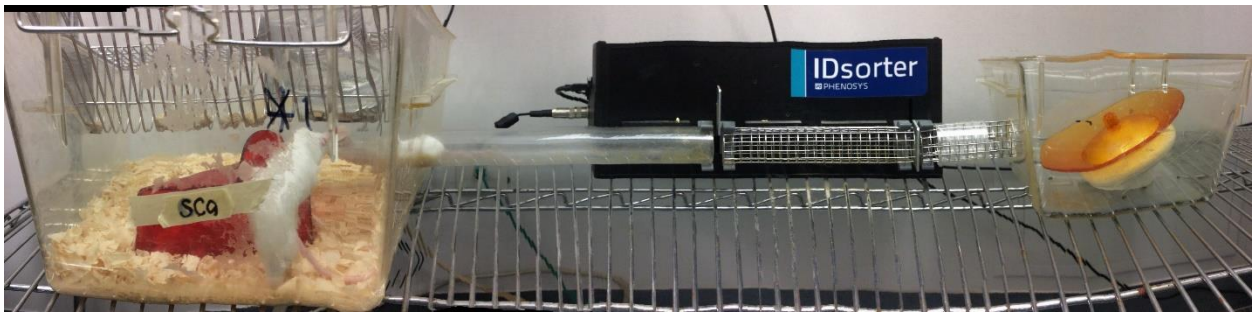

Fig 2: Cage setup. Final experimental setup.

Excess space in the accessory cage was limited by a custom plastic partition, leaving little free area outside of the wheel. This design limited utility of the cage solely to wheel running. The wheels were battery operated and 15cm in diameter. The wheel running software automatically monitored revolutions over time with a resolution of one minute (Med-Associates, St. Albans, Vermont).

Dr. David Coppola and Dr. Waters conducted bulbectomies on CD-1 mice. Mice were allowed to recover from this procedure for at least two weeks before they were paired to

form colonies and wheel access was granted. During this recovery period, mice had RFID tags subcutaneously inserted. We confirmed successful surgical removal of the olfactory bulb with a buried food test at the beginning and end of the experimental period, following a protocol established by Yang and Crawley (2009). In this evaluation, food deprived mice were placed in a cage with 3 cm of bedding. After a five minute period of acclimation, a piece of cereal was buried in a corner of the cage. If mice could not find the food after a period of fifteen minutes, this was taken as a sign of anosmia. If we could not confirm whether a given OBX mouse had fully lost its sense of smell, we excluded it from final analysis. We performed modified SHIRPA physical assessments of the mice prior to the experimentation period to obtain a baseline measure of health. These included documenting weight, observations of physical wellbeing, response to touch and positional changes, and reflex evaluations.

Two rounds of experimentation were conducted, with four colonies of two mice each conducted in one round and an additional four colonies established in the second. Due to attrition and a lack of OBX confirmation in two colonies, the final sample size was six colonies (3 OBX and 3 control). Three additional colonies of two control mice each were provided direct access to a running wheel in the home cage to compare the effect of the RFID gate on wheel running.

Table 1: Experimental Design

OBX Colonies (with surgery and sorter)	Control Colonies (no surgery with sorter)	Free Access Colonies (no sorter)
2 mice	2 mice	2 mice
2 mice	2 mice	2 mice
2 mice	2 mice	2 mice

Two tube tests were conducted over the course of the experimental period to evaluate the social hierarchy of each colony. In a procedure developed by Dr. Waters' lab, seven rounds of dyadic trials are used to calculate a total dominance ratio (TDR) score. This score provides an estimation of each mouse's position in each colony's respective social hierarchy.

Mice had wheel access for a period of three weeks before hormonal data were collected. The first experimental group was immediately sacrificed by cervical dislocation and decapitation, and trunk blood was collected at this three week mark. Fecal samples were collected from the second experimental group at the three week mark to collect hormonal data at this point. This second experimental group than underwent an ABC experiment to probe the impact of the sorter system on wheel running. This testing modified the cost of the sorter system, switching from a 30 second wait period, to three days with a three minute cost, to three days of free access. Following this week of additional testing, fecal samples were taken prior to sacrifice and trunk blood was collected following sacrifice. All samples were frozen for storage prior to analysis. Concentrations of corticosterone from the blood plasma and fecal samples were analyzed with ELISA kits (ENZO ADI-900-097), which read corticosterone levels at 405 nm with comparison to a standard curve. Statistical analysis of wheel running data was conducted using EXCEL and SPSS. We ran repeated measure ANOVAs, using a within-subject factor of hour of day and a between-subject factor of surgery status. Dependent variables were minutes in wheel chamber, minutes running on wheel, wheel revolutions, running speed, and wheel cage entries. We analyzed social dominance and corticosterone concentrations between conditions with t-tests.

IACUC approval from UMW for all procedures was obtained in the fall of 2018.

Results

Circadian differences between OBX and control mice in regards to running patterns were established. Mean minutes in chamber, mean wheel revolutions, mean minutes on wheel, and mean speed all significantly differed by hour of day ($p \leq .001$). Minutes spent in the wheel running chamber, wheel revolutions, and minutes on the running wheel all had statistically significant differences between OBX and control animals. These three variables also had significant differences in circadian patterning when comparing OBX and control animals. Indeed, OBX mice are observed to have a delayed increase in activity in response to the start of their dark period at 0900.

Table 2: Differences in wheel running parameters

	$\bar{x}_{OBX}$	$\bar{x}_{CTR}$	Degrees of freedom	F Statistic	Significance
Does min spent in the wheel chamber differ between OBX and control?	3.1026 minutes/hour	5.0223 minutes/hour	23	1.687	p = 0.0007
Does min spent in the wheel chamber differ between OBX and control and hour of day?	3.1026 minutes/hour	5.0223 minutes/hour	23	7.0	p = 0.029
Do wheel revolutions differ between OBX and control?	116.5713 revolutions	285.3819 revolutions	1	66.150	p = 0.005
Do wheel revolutions differ between OBX and control and hour of day	116.5713 revolutions	285.3819 revolutions	23	2.199	p = 0.002
Does min spent on the running wheel differ between OBX and control?	2.9992 minutes	4.8935 minutes	1	12.672	p = 0.005
Does min spent on the running wheel differ between OBX and control and hour of day?	2.9992 minutes	4.8935 minutes	23	1.863	p = 0.012
Does speed differ between OBX and control?	31/7816 meters/min	44.3733 meters/min	1	4.431	p = 0.062
Do wheel entries differ between OBX and control?	715 entries	494 entries	1	2.053	p = 0.182

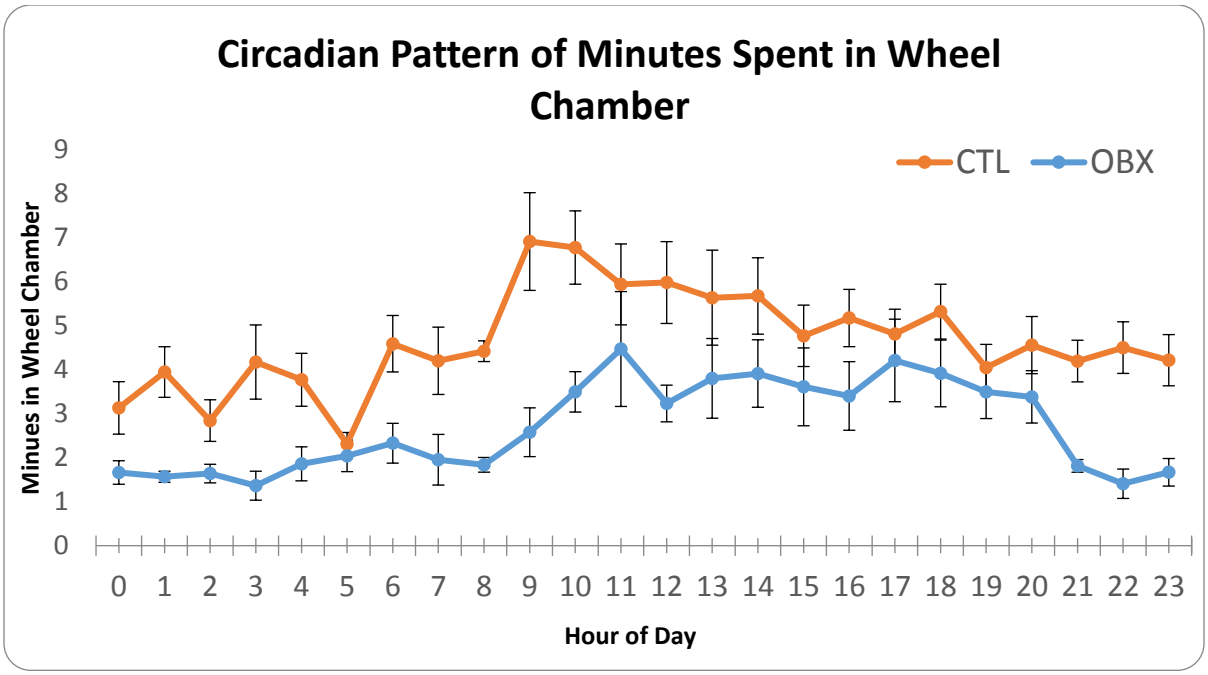

Fig 3: Circadian Patterns. Mean number of minutes spent in the wheel chamber (± SE) by hour between OBX and Control (CTR) mice.

Further circadian differences can be viewed in Figures 11-14 in the Appendices.

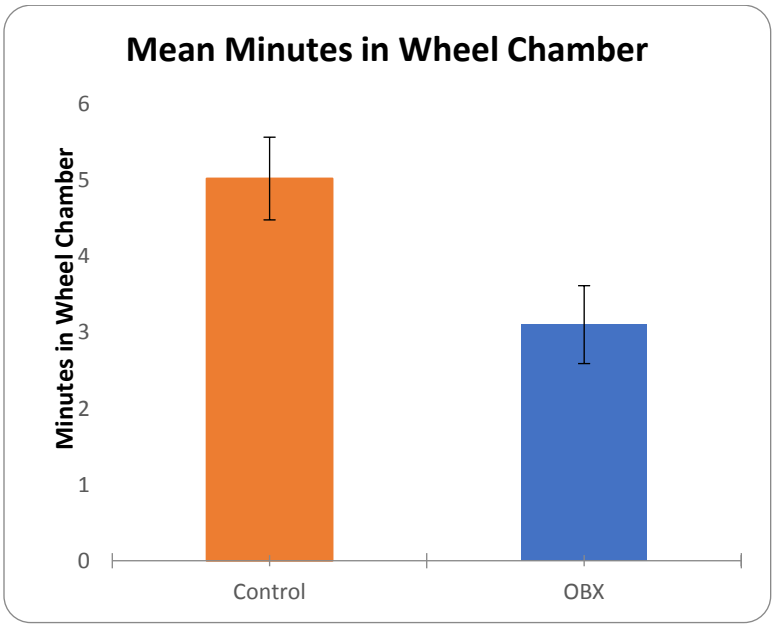

Fig 4: Minutes in wheel chamber. Representation of mean daily totals of minutes spent in the wheel chamber with SE.

Significant differences in running speed between groups were not present, nor were differences in wheel entries. However, the direction of difference in wheel entries differs

from these other wheel running measures, as OBX mice were observed to enter the wheel cage *more* than controls (Fig. 5).

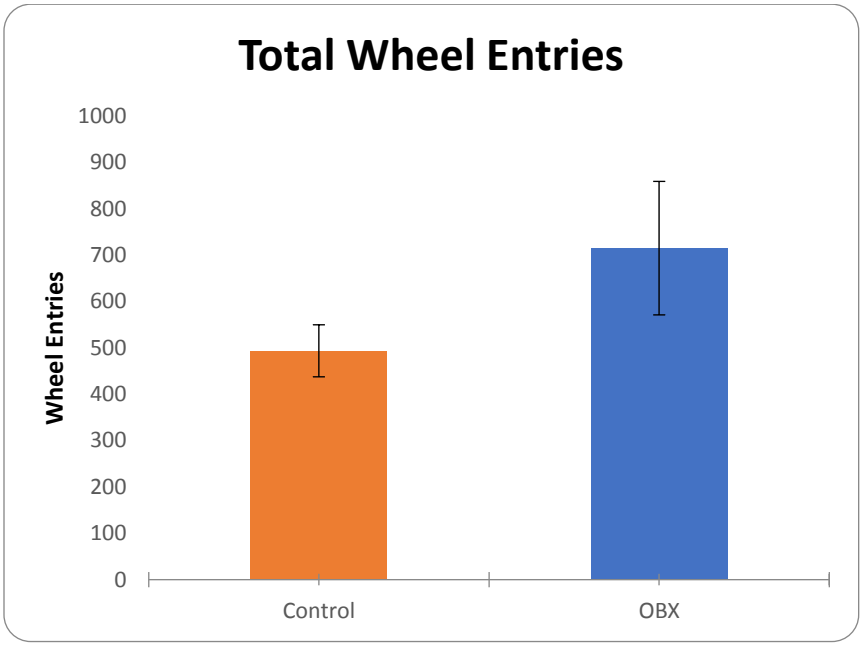

Fig 5: Wheel entries. Mean total wheel entries ± SE are displayed above.

The total dominance ratio scores between bulbectomized and control mice did not significantly differ (Fig 5).

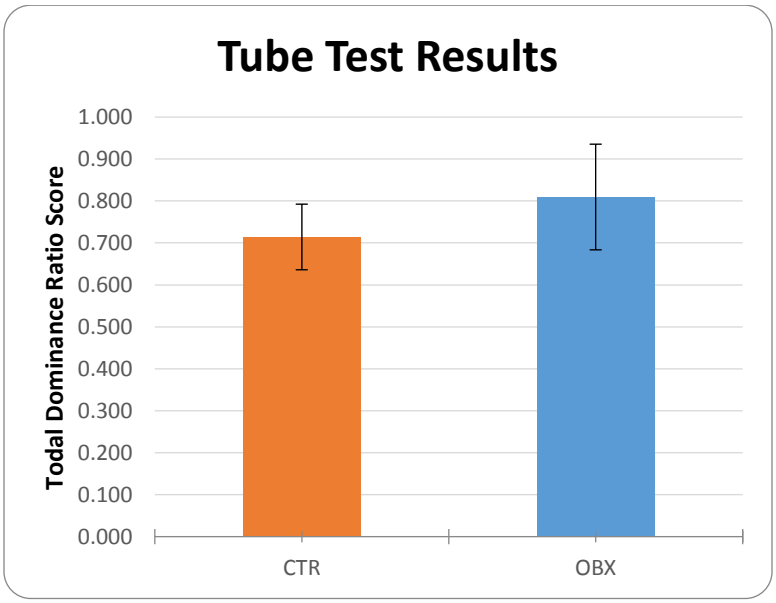

Fig 6: Tube test results. Mean total dominance ratio scores (± SE) for control (CTR) and OBX groups.

Total mean corticosterone concentration of the OBX and control groups were significantly different (t=2.06, df =25, p=0.0438). Fecal corticosterone collected at the three week mark (Fig. 7) showed a difference ($\bar{x}_{CTR}$ = 1.1796 x 10^5, $\bar{x}_{OBX}$ = 2.5945 x 10^5) between the OBX and control groups that approached statistical significance (t= 2.45, df=6, p=0.0612).

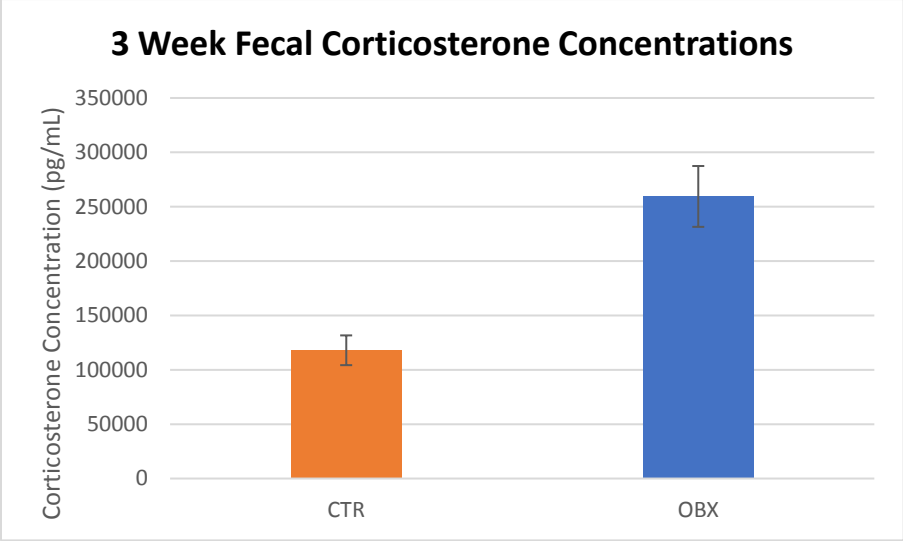

Fig. 7: Fecal corticosterone concentrations at 3 weeks. Mean corticosterone concentrations ± SE are depicted above

These fecal corticosterone differences between the two groups were not maintained at the four week mark (Fig. 8). Interestingly, the direction of difference switched, with control mice demonstrating higher corticosterone concentrations than their OBX counterparts.

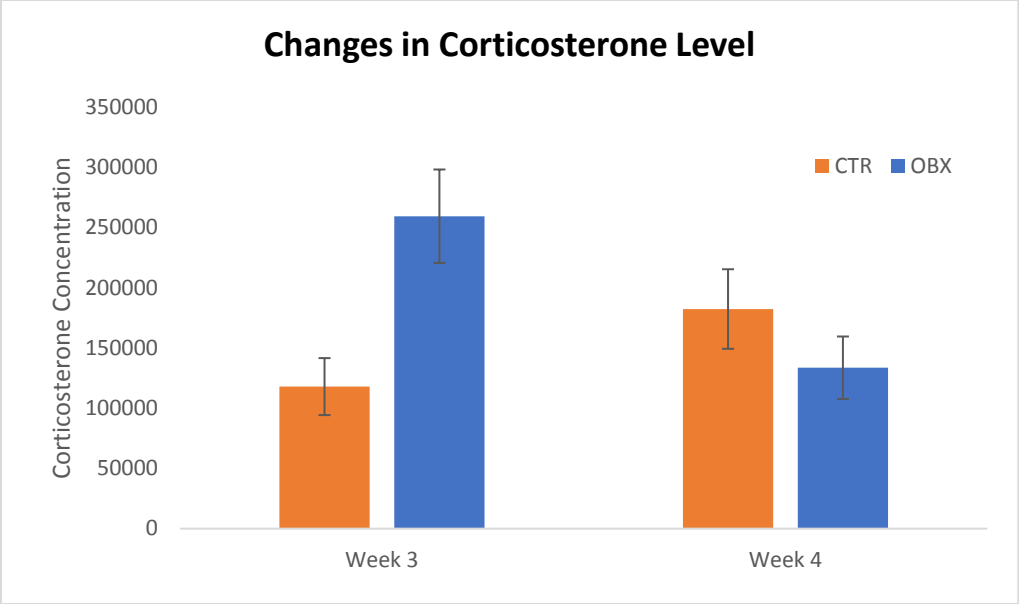

Fig. 8: Changes in corticosterone level between weeks 3 and 4. Mean concentrations ± SE are depicted above

Increasing the wait time to 3 minutes depressed wheel running amongst both OBX and control mice, while providing free access increased wheel running (Fig. 9).

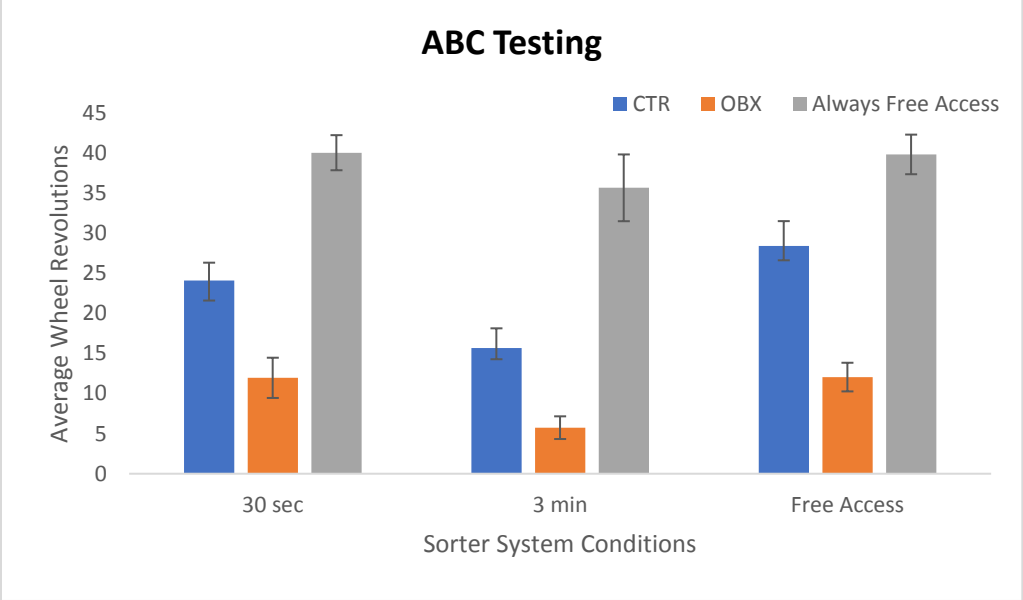

Fig. 9: ABC testing results. Mean wheel revolutions (± SE) for each group under standard, low, and high cost conditions is depicted above.

However, while free access increased wheel running 17.82% among controls (comparing baseline to free access), wheel running only increased 0.79% among OBX mice.

Discussion

Our first hypothesis regarding decreased motivation to run on a wheel in OBX mice is supported. Under experimental conditions that required an operant action to gain wheel access, OBX mice spent less time in the wheel cage, less time on the wheel, and accumulated less wheel revolutions. However, running speed was not significantly affected, nor were number of entries into the wheel cage. In fact, overall number of entries were actually higher in the OBX mice. Higher entries but lower wheel engagement suggests that OBX mice were passively moving between cages, possibly a result of hyperactivity. This may be due to a lack of reinforcement from the act of wheel running, which may have made the more dynamic shuttling between cages a better outlet for hyperactivity. The direction of difference is the opposite of the trend observed in previous research (Rinko and Waters, not published), where OBX mice ran significantly more than controls. This indicates that in our previous free access setup, the higher usage of a running wheel was possibly serving as an outlet for stereotypic motor behavior. Thus, it appears that this new experimental design supports the presence of anhedonia and hyperactivity within the OBX model.

These differences in wheel running activity include differences in circadian patterning. Major differences can clearly be observed in Figure 3 at both 9am and 9pm. These points are particularly significant because they mark the start and end of the dark period. A major spike in activity is seen among the controls when the light is turned off at 9am (the start of the dark period when mice are more active). The delay in OBX activity is indicative of circadian disruption. Circadian rhythm differences in locomotor activity have previously been observed in OBX rodents, signaling a likely connection between the

olfactory bulb and the suprachiasmatic nucleus (Pieper 1991). Our findings support the idea that OBX mice also demonstrate this circadian disruption in our lab.

OBX mice had significantly higher levels of fecal corticosterone after three weeks of wheel running; however, this difference was not observed at the four week level. This difference between the third and fourth week may be due to the last ABC testing condition we used during the last week, which likely impacted hormonal data.

Documented hormonal changes in OBX animals conflict in the literature, with some research finding higher baseline corticosterone concentrations, and others not observing this finding (reviewed in Harkin et al. 2003). Our corticosterone data support the premise that there is an increase in basal corticosterone among OBX mice. Furthermore, it is entirely plausible that the lack of olfaction may itself serve as a stressor that thereby increases corticosterone. Indeed, mice that have genetically induced anosmia demonstrate higher blood plasma corticosterone likely indicative of chronic stress (Glinka et al. 2012). Thus, while the fecal hormone levels themselves may be abnormal, they may actually be indicative of a functioning HPA axis. Another interpretation could be that wheel running behavior reduces anxiety. Voluntary exercise has been established to reduce corticosterone response to stress (Hare et al. 2014). Thus, the controls who ran more may have had their stress attenuated, thereby lowering corticosterone levels.

The differences in the ABC testing conditions further highlight the anhedonic and hyperactive symptoms of OBX mice by demonstrating the cost of the sorter system. The reinforcing nature of wheel running may have contributed to control mice increasing their wheel running when wait times were removed. In contrast, OBX mice had largely negligible changes to wheel running despite this free access. This suggests that these mice did not find

the running wheel to be a reinforcing stimuli. As such, removing barriers to wheel running may not have impacted behaviors if OBX mice were indeed anhedonic.

An aggressive kill in the first round of experimentation among the OBX mice illustrates hyperaggression in the days following surgery. This is a possible behavioral response to bulbectomization not previously documented in literature, which typically has reported passive and docile behavior among male OBX mice (Neckers et. al 1975). In fact, it is speculated that this lack of aggression can be directly linked to a lack of olfaction, which is an important contributor to aggression in other species (reviewed in Harkin et al. 2003), in addition to mice (Ropartz 1968). Thus, the presence of aggression in the course of this experiment indicates that some factor other than olfactory cues must be at play. Furthermore, this novel response was previously observed by Waters and Belanger (not published). Verifying and characterizing this hyperaggressive behavior is a promising area of future research. If this phenotype is a permanent symptom of OBX, it could also feasibly impact wheel behavior by potentially contributing to monopolization of the running wheel by the dominant animal. Determining whether this symptom is transient or permanent would therefore by particularly relevant to better understanding the results of this experiment. Other first round observations include the presence of a white eyed phenotype in three out of four OBX mice. This was present in one or both eyes depending upon the mouse. This may have been the result of eye damage from fighting. If this impaired vision, this could impact anxiety behavior or wheel running behavior directly (indeed, these mice appeared to demonstrate extremes, with some running much more or much less than their control counterparts). As such, these mice were excluded from final analyses.

While this research is promising, it is limited by a small sample size. Repeating this experiment with more colonies may produce more conclusive results. Creating larger colonies, perhaps with three or four mice, may also reveal any nuances in the OBX social hierarchy that a simple dyad could not detect. Perhaps differences may arise among more intermediately dominant mice.

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Appendices

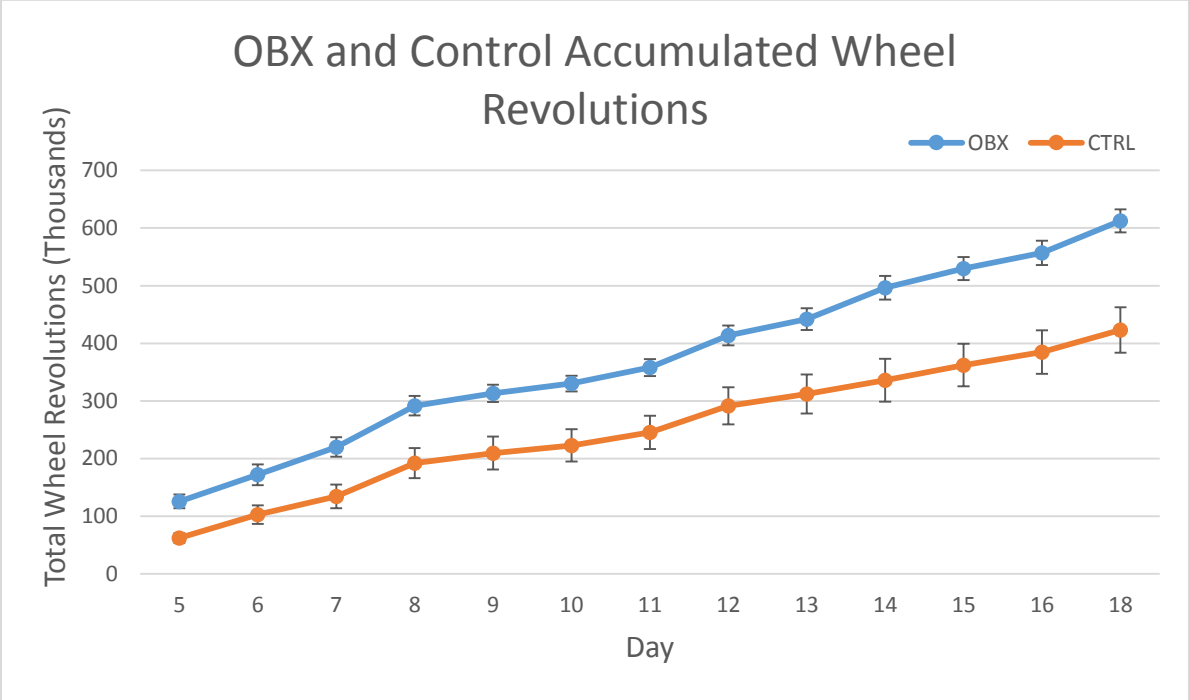

Fig. 10: SSI Results. Under free access conditions OBX mice had statistically significantly higher wheel running that controls (p = 0.022, n=3)

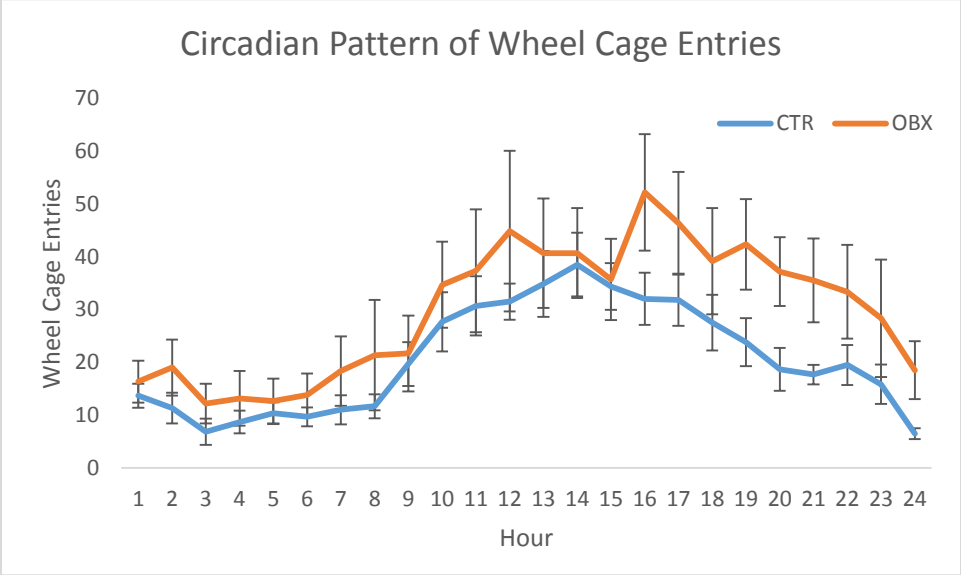

Fig. 11: Circadian Differences in Wheel Entries. Mean number of entries into the wheel chamber ± SEM by hour is depicted above.

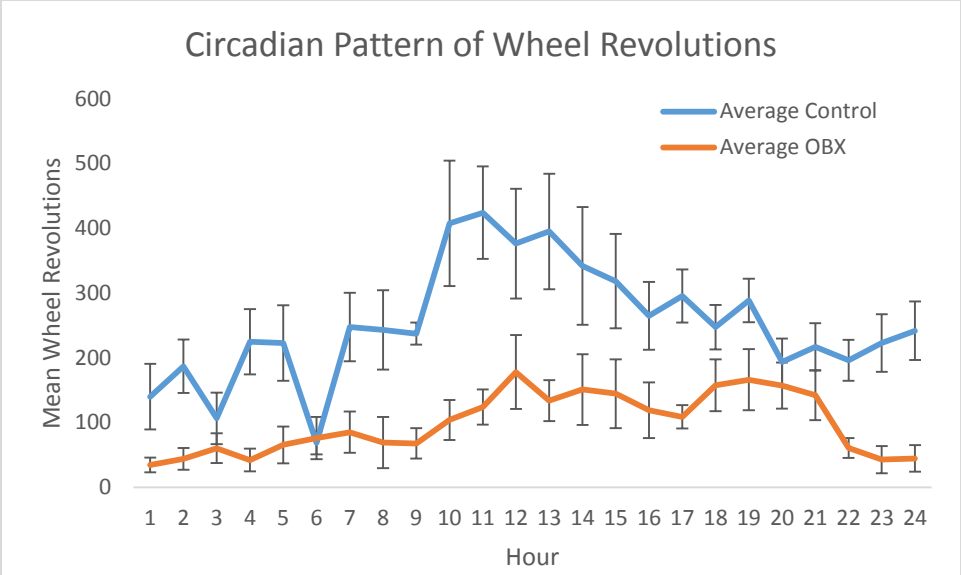

Fig. 12: Circadian Differences in Wheel Revolutions. Mean number of wheel revolutions ± SEM by hour is depicted above.

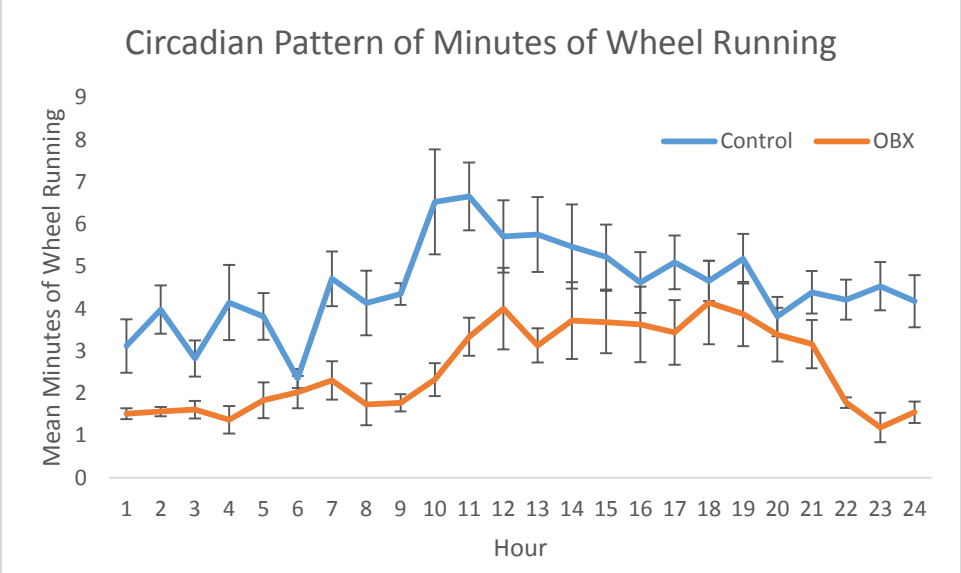

Fig. 13: Circadian Differences in Minutes of Wheel Running. Mean number of minutes of wheel running ± SEM by hour is depicted above.

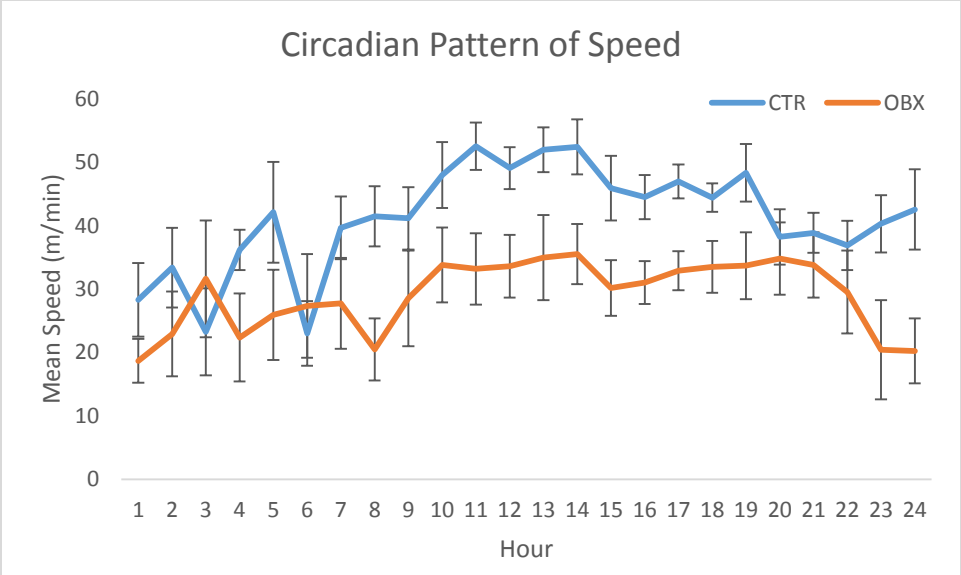

Fig. 14: Circadian Differences in Speed. Mean speed chamber ± SEM by hour is depicted above.