

University at Albany, State University of New York

**Scholars Archive**

---

Biological Sciences

Honors College

---

12-2021

## **Light Level Intensity Alters Anxiety, But Not Memory, during Open-field And Novel Object Location Tasks in Male Rats**

Angelina Tassone

The University at Albany community has made this article openly available.

**Please share** how this access benefits you.

Follow this and additional works at: [https://scholarsarchive.library.albany.edu/honorscollege\\_biology](https://scholarsarchive.library.albany.edu/honorscollege_biology)



Part of the [Biology Commons](#)

---

**Light Level Intensity Alters Anxiety, But Not Memory,  
during Open-field And Novel Object Location Tasks in Male Rats**

An honors thesis presented to the  
Department of Biological Sciences,  
University at Albany, State University of New York  
in partial fulfillment of the requirements  
for graduation with Honors in Biology  
and  
graduation from The Honors College

Angelina R. Tassone

Research Mentor: Corey J. Frank, B.S.  
Research Advisor: Ewan C. McNay, Ph.D.  
Second Reader: Annalisa Scimemi, Ph.D.

December 2021

## Abstract

Appropriate design and control of testing conditions during assessment of animal behavior is critical to maximize generalizability, replicability, and translational relevance. Some sensory stimuli are often controlled: for instance, during rodent behavioral testing, efforts are commonly made to reduce or eliminate olfactory and auditory distractions. However, less attention is paid to the ambient light level intensity (lux), which may vary even between rooms in the same facility. We sought to explore whether behavior is influenced by the standard illumination intensity in one of our behavioral testing rooms. To this end, we measured anxiety-like behaviors, exploration, and spatial memory performance in 7-month-old, male, Sprague-Dawley rats under conditions of either the standard bright light of the testing room (~618 lux) or dim light (~10 lux). During the open-field and novel object location tasks (OFT and NOL, respectively), rats in the bright light condition froze more often and spent more time frozen than rats in the dim condition. In addition, in the OFT with bright illumination, rats spent more time in the corners of the apparatus. No differences were detected in overall mobility or total time spent in the center of the OFT and in object preference or mobility in the NOL with varying levels of illumination. We conclude that in these rats, bright light increases freezing behaviors without altering overall mobility or spatial working memory performance. Our data confirm the importance of measuring, reporting, and controlling lux in experiments measuring rodent behavior.

**Keywords:** *Lux, Open field test, Novel object location test, Anxiety, Memory*

## **Acknowledgements**

I would like to thank Dr. Ewan McNay for his guidance and support throughout my time in the lab that encouraged me to gain a deep appreciation and passion for science.

I would like to thank Dr. Annalisa Scimemi for graciously agreeing to act as the second committee member for this thesis.

I would like to extend a great thank you to Corey Frank for his extensive mentorship throughout my time in the lab and especially during this study – including guidance and assistance during the literature search, experiments, data analysis, and interpretation of findings. Without his support, I would not have been able to complete this thesis.

I would like to thank the other graduate students in the McNay lab, Starlette Douglass and Greg Fitzgerald for their support and mentorship throughout my time in the lab.

I would like to extend a great thank you to McNay lab members Alayna Stickles, Teja Buddhavarapu, and Jose Velez for their assistance in behavioral scoring and data preparation. A warm thank you goes to all the other McNay lab members. I am grateful to have received support from each and every single one of you.

Lastly, I would like to thank my family and friends for their constant love, encouragement, and support. I would not be where I am today without you all.

## List of Figures

<b>Figure 1.</b> OL testing in different lighting conditions .....	7
<b>Figure 2.</b> Counterbalancing of target and familiar object locations in NOL .....	8
<b>Figure 3.</b> Freezing behavior during OFT is dependent on light level .....	10
<b>Figure 4.</b> Anxiety-like behavior during OFT is dependent on light level.....	11
<b>Figure 5.</b> Freezing behavior during NOL is dependent on light level .....	12

## List of Tables

<b>Table 1.</b> Possible confounds in rodent behavioral testing .....	2
---	---

## Table of Contents

<b>Abstract</b> .....	ii
<b>Acknowledgements</b> .....	iii
<b>List of Figures</b> .....	iv
<b>List of Tables</b> .....	v
<b>Introduction</b> .....	1
<b>Materials and Methods</b>	
Ethics statement .....	6
Animals and acclimatization .....	6
Apparatus and lighting .....	6
OFT and NOL .....	8
AnyMaze and rater scoring .....	9
<b>Results</b>	
OFT .....	10
NOL .....	11
<b>Discussion</b> .....	13
<b>References</b> .....	16

## Introduction

Differences in the testing environment may be critical for shaping animal behavior, as they can influence the validity and generalizability of the results. As summarized in a recent review (Saré et al., 2021), there are many potential confounding factors that should be considered before running experimental trials, to ensure replicability both within the same lab and between labs (**Table 1**). It is important to keep the testing site within the same lab and individual studies consistent. Anxiety-like behaviors in mice differed within the same laboratory when the testing location changed (Wahlsten et al., 2006). In the housing facility and testing site, noise from heavy personnel traffic increased corticosterone levels in laboratory animals (Rabat, 2007). Handling can also influence animal behavior and therefore results of behavior assays. Higher overall mobility was observed in both Sprague-Dawley and PVG/OlaHsd rats that were handled before testing as compared to strain-matched rats that were not handled (Schmitt & Hiemke, 1998). The experimenter identity should remain consistent, as handling techniques can differ. The experimenter was the largest influence on tail-withdrawal latencies following hot water submersion, and this effect was not caused by the sex or age of the experimenter (Chesler et al., 2002).

Any vulnerability or resilience to environmental variables can magnify or obscure true effects, therefore increasing error rate and reducing accuracy of behavioral tests. This is important, as behavioral assays often display high variability and small effect sizes. For instance in the novel object recognition (NOR) test, the mean preference score for the novel object is 60-70% for healthy animals, and 50% for equal exploration time of the novel and familiar objects (which would be



interpreted as showing no memory of the familiar object), meaning that even moderate effects of extraneous factors may increase the likelihood of erroneous conclusions (Gulinello et al., 2019).

**Table 1**  
*Possible confounds in rodent behavioral testing*

Time of day
Testing site
Auditory stimuli
Olfactory stimuli
Light level
Experimenter
Handling
Order of tests
Spacing between tests
Housing density

*Note.* These factors can act as confounding variables if not properly controlled for, therefore, affecting the replicability and generalizability of the experimental results. While auditory and olfactory stimuli are often controlled for, light level is often not.

Without consensus, research has explored the impact of light level on assays of anxiety, for instance the elevated plus maze (EPM) and OFT. Several labs have found that in rats, higher lux levels are associated with increased anxiety-related behaviors in an OFT (Bouwknicht et al., 2007; Hughes et al., 2014; Miller et al., 2021) and EPM (Garcia et al., 2011; Kapogiannatou et al., 2016). Similar results for OFT were also observed in mice (Martin-Arenas & Pintado, 2019). These data are consistent with rodent preference for dimly-lit areas, which is the reason why several behavioral tasks such as inhibitory avoidance (Atucha & Roozendaal, 2015) and the light-dark box (LDB; Bourin & Hascoët, 2003) are performed in dim-light conditions. However,

prior results found no difference in EPM performance in differently lit environments in rats (Becker & Grecksch, 1996) and mice (Shoji & Miyakawa, 2021), suggesting a need for further study.

Moreover, less attention has been focused on the impact of light level on commonly used assays of working memory, despite the known relationship between other stressors and memory performance (Ma et al., 2007). Nile grass rats showed impaired spatial memory in a Water Maze (WM) when tested in dim light, but performance was regained if the rats were transferred to the bright condition (Soler et al., 2018). In contrast to these results, BALB/c mice performed well under the dim condition in WM, but could not complete the task in bright conditions, when their corticosterone levels increased (Huang et al., 2012). Bright light during the familiarization phase of NOR is required for the formation of long-term, but not short-term memories (Moore et al., 2013). The same study found significantly altered plasma corticosterone levels between dim/low conditions, but these differences did not impact eventual testing performance (Moore et al., 2013). Results concerning the impact of lux on locomotor activity are likewise mixed. Reduced locomotion due to bright light has been observed (Godsil & Fanselow, 2004) while other experimenters have found no difference in locomotion (Garcia et al., 2005; Kapogiannatou et al., 2016). The impact of lux on locomotion may also be dependent on sex, as reduced locomotion has been observed in female rats but not males (Miller et al., 2021).

There are significant rodent strain differences regarding vulnerability to stress and expression of anxiety-like behaviors (Ramos et al., 1997). Comparison of OFT behavior between Wistar and Fawn Hooded rats found that the strains respond differently to lighting conditions during testing (Hall et al., 2000). Lewis rats and spontaneous hypertensive rats exhibit both strain and sex differences in several measures of anxiety-like traits (Ramos et al., 1998, 2002). On the

elevated plus maze, older rats tested towards the end of the light period performed worse than younger rats and older rats tested earlier in the light period, even given the same inter-trial interval (Morales-Delgado et al., 2018) and in a measure of exploratory behaviors in the EPM, the impact of bright vs dim light likewise depended on the age of the rat and the time of day of testing (Albani et al., 2015). Even given the same rodent age, sex, and housing/testing environment, rats obtained from different vendors exhibit different performance on OFT and LDB testing (Tsuda et al., 2020). Circadian factors and apparatus details, such as color, can also impact performance. In OFT, mice tested in an arena with a black floor tended to display more exploratory behaviors and reduced anxiety (Kuleskaya & Voikar, 2014). The sensitivity to lighting conditions during behavioral tasks is likely based on the nocturnal nature of many species of rodents. In Long-Evans rats, sleep occupies 80% of daytime hours (Frank et al., 2017) and in Wistar rats a similar percentage of food consumption occurs during the nighttime (Sidlo et al., 1995). However, most behavioral assays are performed during the light cycle (Aslani et al., 2014), often due to issues of experimental feasibility. These studies suggest that environmental conditions can alter behavior differently depending on other variables. While age, sex, strain, and time of testing are typically reported, we argue that ambient environmental factors should likewise be reported.

Here, we compared behavior on OFT and NOL under the standard illumination levels of an animal testing room at  $618 \pm 2$  lux with behavior in a low,  $10 \pm 0.2$  lux illumination. The goal of this experiment was to determine whether the light level of our testing room would impact performance on two common behavioral tasks. Illumination was sufficient to allow the rat's behavior to be captured by a standard  $1080 \times 720$ p webcam (C615, Logitech, Lausanne, Switzerland) and tracked in AnyMaze software (version 7.08, Stoelting Co., Wood Dale IL,

60191). The OFT is a well validated assessment of anxiety-like behaviors that relies upon the balance between a rat's tendency to spontaneously explore its environment and the innate desire to stay near walls or vertical sections (thigmotaxis; Bouwknecht et al., 2007; Cunha & Masur, 1978; Prut & Belzung, 2003). OFT is sensitive to pharmacological, environmental, and genetic manipulation (Bronikowski et al., 2001; Choleris, 2001; Rojas-Carvajal et al., 2018). The NOL task is a variant of the novel object recognition task where the target object is moved to a new location within the apparatus between the learning and testing trials. NOL relies on the innate curiosity of the rat to explore changed aspects of the environment (Vogel-Ciernia & Wood, 2014). NOL is an effective assay of spatial learning and memory performance and is heavily reliant upon hippocampal processing (Denninger et al., 2018; Mumby et al., 2002; Poulter et al., 2020), making it a useful test in animal models of hippocampally associated diseases like Alzheimer's Disease. NOL can be performed without the need for extensive pre-test training (Vogel-Ciernia & Wood, 2014), with different inter-trial intervals, and at a low cost (Denninger et al., 2018), making it a versatile and flexible behavioral assay.

## Materials and Methods

### Ethics statement

All procedures were approved by the University at Albany Institutional Animal Care and Use Committee (IACUC).

### Animals and acclimatization

All experiments were performed on 37-38 week old male Sprague Dawley rats, CD substrain (Charles River Breeding Laboratories, Wilmington, MA 01887). Prior to behavioral testing rats were handled for 10 minutes per day for 7 days in the same room as behavioral testing under the bright/ambient light conditions, in order to familiarize the rats to the experimenter and the testing room. Rats were naive to the behavior assays used in this experiment but had been assessed for working memory in a different apparatus approximately 4 months prior. Rats were pair housed throughout the entire experiment, given *ad libitum* access to standard lab chow and water, and kept on a 12:12 light:dark cycle (lights on at 7 am). All testing was performed during the day cycle between 9:30 am and 2:00 pm. Each cage of pair-housed rats had one rat assigned to the dim-light condition and one rat assigned to the bright-light condition. Testing order was counterbalanced to account for any potential effect caused by the brief removal of their cage-mate.

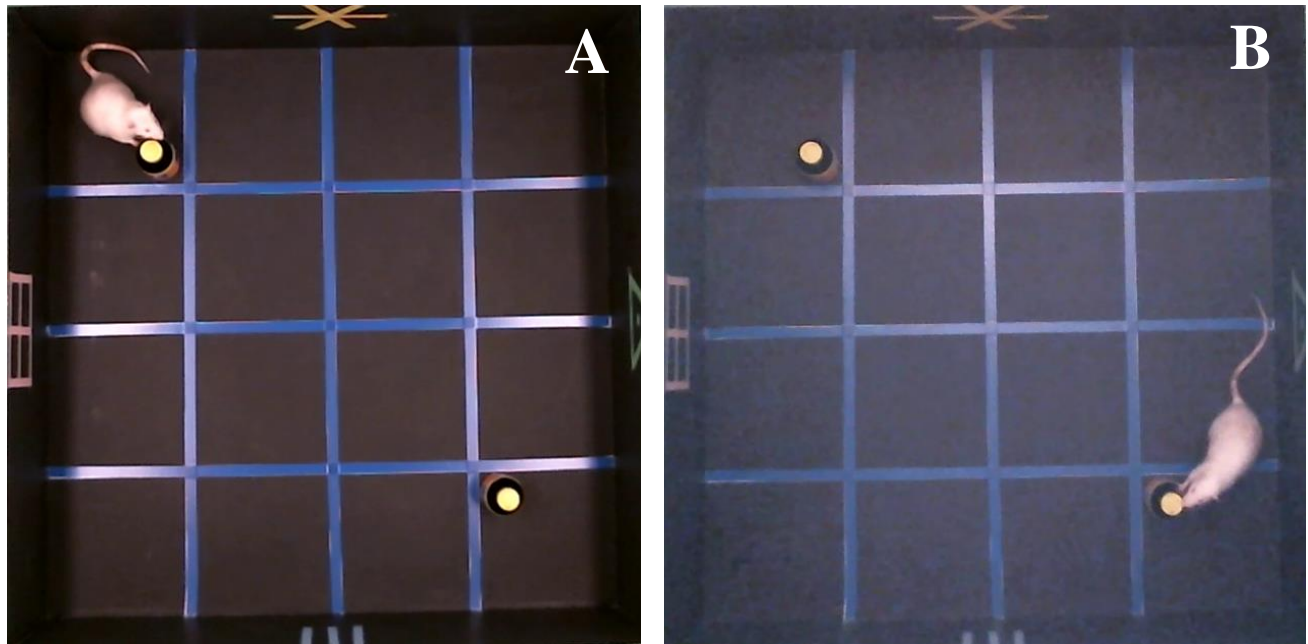
### Apparatus and lighting

The testing apparatus consisted of a matte-black plexiglass box 100 cm × 100 cm × 30cm (L × W × H). Target objects for NOL consisted of two identical 250 ml Wheaton bottles filled with bright blue liquid and festooned with colored tape. The 618 lux light for the bright condition (**Fig. 1A**) was produced by overhead fluorescent bulbs and light for the dim, 10 lux condition (**Fig. 1B**) was produced by a single overhead 40-Watt bulb attached to a dimmer. Daily, before training or testing, lux was measured at the center of the apparatus via handheld lux meter (LX1330B, Dr.

Meter, Newark, CA 94560) to ensure intensity remained at the predetermined lux level. The apparatus was thoroughly cleaned with 70% ethanol between trials to eliminate any residual odors. Rat behavior was recorded via an overhead camera connected to a laptop directly outside the testing room. Prior to testing, rats were kept in a closed hallway outside the testing room and given a minimum of 30 minutes after transport before testing. Then, 5 minutes prior to the start of behavioral training or testing, rats were moved into a holding cage in the testing room and allowed to acclimate to the experimental lighting condition.

### Figure 1

*NOL testing in different lighting conditions*



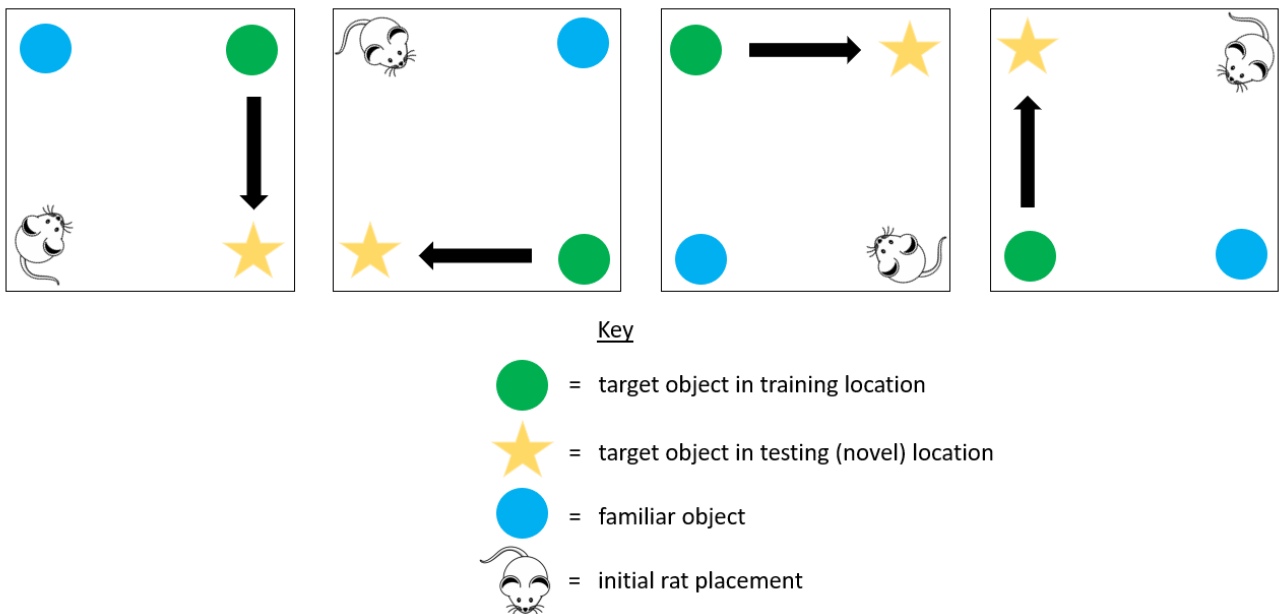
*Note.* NOL apparatus in the bright (*left*) and dim (*right*) conditions. The placement of the novel and familiar objects was counterbalanced across trials and subjects.

## OFT and NOL

During OFT, rats were placed in the center of the apparatus, recorded for 5 minutes, and then returned to their home cage. One day following OFT, rats were trained in NOL in the same apparatus, in the same room, under the same lighting conditions. During training, both objects were placed on either corner of the same wall and rats were placed on the opposite side of the apparatus in corner diagonal from the target object. Configuration of the bottles and placement of the rats within the apparatus were counterbalanced between conditions to account for any potential biases. Behavior was recorded for 5 minutes and then each rat was returned to its home cage. Light level, pre-trial acclimation period, and objects were the same as those used during NOL training. The target object was moved to the corner diagonally opposite from the familiar object and the rat was placed in the same corner of the apparatus as during training. Following completion of NOL testing rats were removed to their home cage and returned to the colony room.

**Figure 2**

*Counterbalancing of target and familiar object locations in NOL*



*Note.* The placement of the target and familiar object locations was counterbalanced across trials and subjects. The target object was moved to the other corner of the same wall for the testing phase.

### **AnyMaze and rater scoring**

OFT and NOL performance was measured automatically with AnyMaze software by raters uninvolved with the original experiment. Test videos of non-experimental animals were analyzed first to ensure that the rats could still be seen by the camera in sufficient clarity to allow for behavioral scoring in AnyMaze. During analysis, raters manually scored rearing behaviors in both OFT and NOL and manually scored object interaction in NOL. Three raters scored every video for OFT and two raters scored every video for NOL, with the mean taken of all scores to be used for analysis. While blinding to the independent variable was not possible, raters were blind to the location of the novel and familiar object in the apparatus. Primary behavior measures for OFT were overall mobility, time spent and entries into the center 4 squares, time spent and entries into the 4 corner squares, time spent and entries into the peripheral squares along the walls, episodes of and time spent freezing, and episodes and time spent rearing. Primary behavior measures for NOL were overall mobility, time spent exploring the novel vs familiar object, discrimination ratio between the novel and familiar object, episodes of and time spent freezing, and episodes of and time spent rearing. During OFT one rat broke a nail and was excluded from all later analysis.



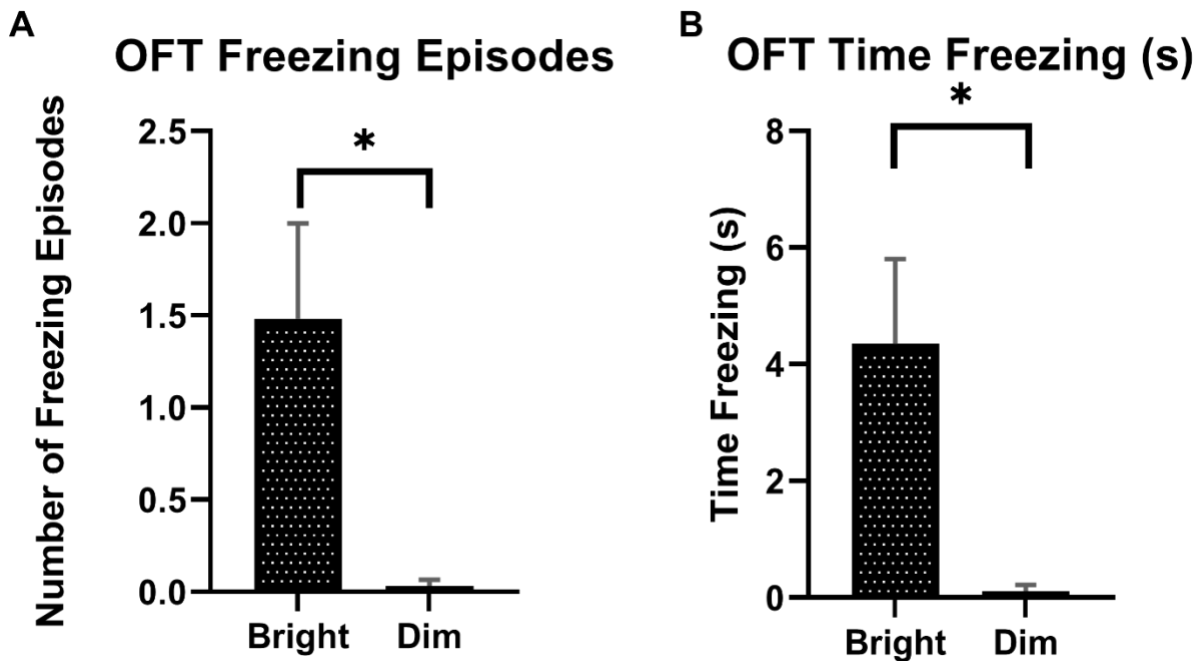
## Results

### OFT

Levene's test for assumption of normality was violated for time spent and episodes of freezing, therefore the non-parametric Mann-Whitney U test was used to compare group distributions. Student's T-test was used for all other analyses. Rats in bright-light condition froze more frequently ( $U = 73$ ,  $n_1 = 9$ ,  $n_2 = 10$ ,  $* p = 0.02$ ; **Fig. 3A**), spent more time frozen ( $U = 73.5$ ,  $n_1 = 9$ ,  $n_2 = 10$ ,  $* p = 0.02$ ; **Fig. 3B**), and spent more time with their heads within the corners of the apparatus ( $T = 2.229$ ,  $df = 17$ ,  $* p = 0.04$ ; **Fig. 4**). No significant differences were detected in overall distance travelled or time spent in the center zone.

**Figure 3**

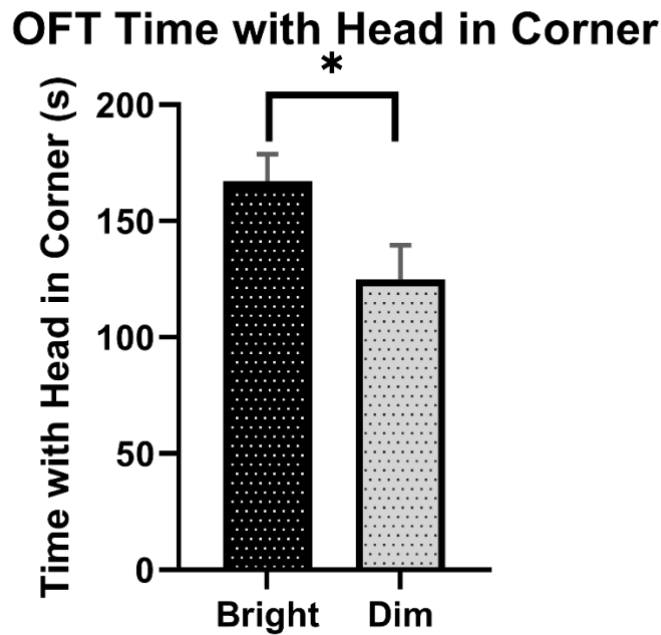
*Freezing behavior during OFT is dependent on light level*



*Note.* (A) Rats in the bright light condition froze more frequently and (B) spent more time freezing. Error bars represent standard error of the means (SEM).  $* p < 0.05$ .

#### Figure 4

*Anxiety-like behavior during OFT is dependent on light level*



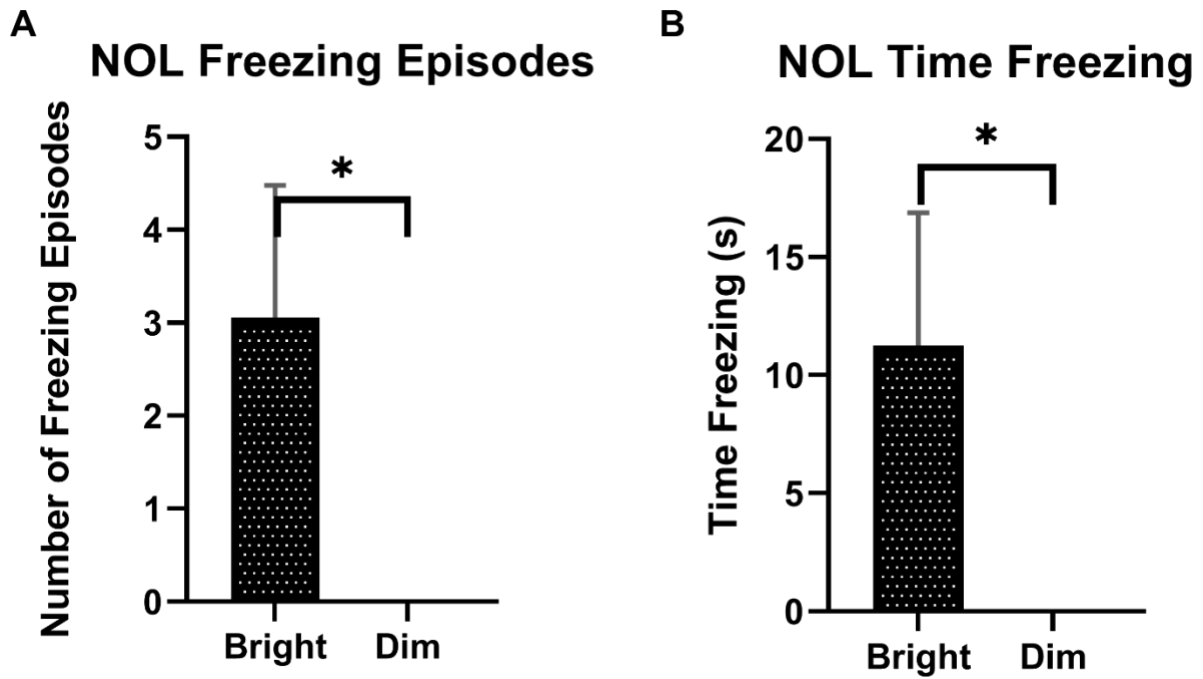
*Note.* Rats in the bright light condition spent more time with their head in the corner during OFT. Error bars represent SEM. \* $p < 0.05$ .

#### NOL

Levene's test for assumption of normality was violated for time spent and episodes of freezing, therefore the Mann-Whitney U test was used to compare group means. Rats in the bright-light condition froze more frequently ( $U = 20, n_1 = 9, n_2 = 10, *p = 0.043$ ; **Fig. 5A**) and spent longer frozen ( $U = 20, n_1 = 9, n_2 = 10, *p = 0.043$ ; **Fig. 5B**). No significant differences were detected in overall distance travelled, time spent exploring the novel or familiar objects, or discrimination ratio between the novel and familiar object.

**Figure 5**

*Freezing behavior during NOL is dependent on light level*



*Note.* (A) Rats in the bright light condition froze more frequently and (B) spent more time freezing. Error bars represent standard error. \*  $p < 0.05$ .

## Discussion

Our results show that bright ambient illumination increases anxiety-like behaviors in male rats. Accordingly, rats tested in bright light conditions displayed increased freezing behaviors and preference towards staying in the corners of an open field. Further, the anxiogenic effects of bright lighting conditions persisted between OFT and NOL. However, despite the apparent anxiogenic effect of the brighter environment, no difference was detected in overall mobility in either test, nor any difference in object preference and exploration in NOL. This may be because the bright light was not sufficiently stressful to interfere with memory encoding. From raters' observations of the trials, it is also possible that rats in each condition were acclimating to the light conditions during the trial, so our data may represent the speed and manner of acclimation to the light intensity while obscuring differences in memory performance. Alternatively, movements in bright conditions may tend to be faster, albeit being interspersed with periods of immobility. Future studies will examine trials broken up into timepoints to determine if acclimation is indeed influencing behavioral outcomes. If so, a longer pre-trial acclimation period can be used to minimize this effect.

This study has several important limitations. Our experiments were only performed in male rats of a particular strain (Sprague-Dawley), within a narrow age range. These rats were tested at just two light levels based on previous literature and conditions within the testing facility. As sex differences exist for other aversive stimuli, such as pain sensitivity (Vierck et al., 2008), it is reasonable to assume that sex differences may likewise exist for the anxiogenic potential of bright light. Further work would be required to determine whether sex, strain, and age effects exist. Different spectra of light, such as red light, can also be investigated. Likewise, wider extremes of light levels may impact behavior, although many of these values would not be found in typical

testing environments. Future experiments would benefit from monitoring molecular measures of stress before and after the behavioral task or from expanding the behavioral tasks of interest.

It is also possible to incorporate environmental variables into the design of behavioral assays to expand the validity and increase sensitivity. A potential solution to the possible confound of bright illumination is to integrate light level into the design of the task, such as hybrid behavioral tasks that combine aspects of OFT and LDB (Shanazz et al., 2021) and EPM (Ramos et al., 2008). Light-dark open-field increases test discrimination and raises the ceiling for detection of anxiety-like behaviors in both Sprague Dawley and high-anxiety Lewis rats by incorporating brightly lit and shadowed areas into a standard OFT apparatus (Shanazz et al., 2021). Increasing the types of environments accessible during behavioral assessment can likewise integrate light level into the overall design. Ramos et al. (2008) examined the use of a hybrid apparatus that connected an open-field arena to an elevated plus maze (via the closed arms) and then onto the dark chamber of a light-dark box. This model allows for simultaneous assessment of multiple measures of anxiety-like behaviors such as exploration (center area in OFT or open arms in EPM), thigmotaxis (peripheral time in OFT or closed-arm time in EPM) and aversion to brighter illumination (center area in OFT, open arms in EPM, light section in LDB). By integrating lighting conditions into the overall design of the assay, experimenters can minimize confounds and increase the quality of their behavioral data.

In sum, awareness of and accounting for potential environmental cues and stimuli is an important aspect of experimental design. In the case of light intensity, behavioral changes that may be apparent under dim light may be suppressed under bright testing conditions, potentially reducing the sensitivity of behavioral assays and obscuring genuine behavioral effects. For this reason, experimenters must endeavor to consider potential sensory experiences from the subject's

perspective. While traditional experimental wisdom holds that extraneous factors of an experiment are mitigated by keeping such factors consistent across experimental groups, this rationale only applies if the extraneous factor has a consistent effect across groups. For variables that can be considered stressors, there may be group differences in the vulnerability or resilience to such stressors, confounding potential results. Therefore, every effort should be made to minimize the influences of such variables.

## References

- Albani, S. H., Andrawis, M. M., Abella, R. J. H., Fulghum, J. T., Vafamand, N., & Dumas, T. C. (2015). Behavior in the elevated plus maze is differentially affected by testing conditions in rats under and over three weeks of age. *Frontiers in Behavioral Neuroscience, 9*.  
<https://doi.org/10.3389/fnbeh.2015.00031>
- Aslani, S., Harb, M. R., Costa, P. S., Almeida, O. F. X., Sousa, N., & Palha, J. A. (2014). Day and night: Diurnal phase influences the response to chronic mild stress. *Frontiers in Behavioral Neuroscience, 8*. <https://doi.org/10.3389/fnbeh.2014.00082>
- Atucha, E., & Roozendaal, B. (2015). The inhibitory avoidance discrimination task to investigate accuracy of memory. *Frontiers in Behavioral Neuroscience, 9*.  
<https://doi.org/10.3389/fnbeh.2015.00060>
- Becker, A., & Grecksch, G. (1996). Illumination has no effect on rats' behavior in the elevated plus-maze. *Physiology & Behavior, 59*(6), 1175–1177. [https://doi.org/10.1016/0031-9384\(95\)02224-4](https://doi.org/10.1016/0031-9384(95)02224-4)
- Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology, 463*(1–3), 55–65. [https://doi.org/10.1016/s0014-2999\(03\)01274-3](https://doi.org/10.1016/s0014-2999(03)01274-3)
- Bouwknicht, J. A., Spiga, F., Staub, D. R., Hale, M. W., Shekhar, A., & Lowry, C. A. (2007). Differential effects of exposure to low-light or high-light open-field on anxiety-related behaviors: Relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. *Brain Research Bulletin, 72*(1), 32–43.  
<https://doi.org/10.1016/j.brainresbull.2006.12.009>

- Bronikowski, A. M., Carter, P. A., Swallow, J. G., Girard, I. A., Rhodes, J. S., & Garland, Jr., T. (2001). Open-field behavior of house mice selectively bred for high voluntary wheel-running. *Behavior Genetics*, *31*(3), 309–316. <https://doi.org/10.1023/A:1012283426530>
- Chesler, E. J., Wilson, S. G., Lariviere, W. R., Rodriguez-Zas, S. L., & Mogil, J. S. (2002). Influences of laboratory environment on behavior. *Nature Neuroscience*, *5*(11), 1101–1102. <https://doi.org/10.1038/nn1102-1101>
- Choleris, E. (2001). A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neuroscience & Biobehavioral Reviews*, *25*(3), 235–260. [https://doi.org/10.1016/S0149-7634\(01\)00011-2](https://doi.org/10.1016/S0149-7634(01)00011-2)
- Cunha, J. M., & Masur, J. (1978). Evaluation of psychotropic drugs with a modified open field test. *Pharmacology*, *16*(5), 259–267. <https://doi.org/10.1159/000136777>
- Denninger, J. K., Smith, B. M., & Kirby, E. D. (2018). Novel object recognition and object location behavioral testing in mice on a budget. *Journal of Visualized Experiments*, *141*. <https://doi.org/10.3791/58593>
- Frank, M. G., Ruby, N. F., Heller, H. C., & Franken, P. (2017). Development of Circadian sleep regulation in the rat: A longitudinal study under constant conditions. *Sleep*, *40*(3). <https://doi.org/10.1093/sleep/zsw077>
- Garcia, A. M. B., Cardenas, F. P., & Morato, S. (2005). Effect of different illumination levels on rat behavior in the elevated plus-maze. *Physiology & Behavior*, *85*(3), 265–270. <https://doi.org/10.1016/j.physbeh.2005.04.007>



- Garcia, A. M. B., Cardenas, F. P., & Morato, S. (2011). The effects of pentylenetetrazol, chlordiazepoxide and caffeine in rats tested in the elevated plus-maze depend on the experimental illumination. *Behavioural Brain Research*, *217*(1), 171–177.  
<https://doi.org/10.1016/j.bbr.2010.09.032>
- Godsil, B. P., & Fanselow, M. S. (2004). Light stimulus change evokes an activity response in the rat. *Animal Learning & Behavior*, *32*(3), 299–310.  
<https://doi.org/10.3758/BF03196029>
- Gulinello, M., Mitchell, H. A., Chang, Q., Timothy O'Brien, W., Zhou, Z., Abel, T., Wang, L., Corbin, J. G., Veeraragavan, S., Samaco, R. C., Andrews, N. A., Fagiolini, M., Cole, T. B., Burbacher, T. M., & Crawley, J. N. (2019). Rigor and reproducibility in rodent behavioral research. *Neurobiology of Learning and Memory*, *165*, 106780.  
<https://doi.org/10.1016/j.nlm.2018.01.001>
- Hall, F. S., Huang, S., Fong, G. W., Sundstrom, J. M., & Pert, A. (2000). Differential basis of strain and rearing effects on open-field behavior in Fawn Hooded and Wistar rats. *Physiology & Behavior*, *71*(5), 525–532. [https://doi.org/10.1016/S0031-9384\(00\)00372-3](https://doi.org/10.1016/S0031-9384(00)00372-3)
- Huang, Y., Zhou, W., & Zhang, Y. (2012). Bright lighting conditions during testing increase thigmotaxis and impair water maze performance in BALB/c mice. *Behavioural Brain Research*, *226*(1), 26–31. <https://doi.org/10.1016/j.bbr.2011.08.043>
- Hughes, R. N., Hancock, N. J., Henwood, G. A., & Rapley, S. A. (2014). Evidence for anxiolytic effects of acute caffeine on anxiety-related behavior in male and female rats tested with and without bright light. *Behavioural Brain Research*, *271*, 7–15.  
<https://doi.org/10.1016/j.bbr.2014.05.038>

- Kapogiannatou, A., Paronis, E., Paschidis, K., Polissidis, A., & Kostomitsopoulos, N. G. (2016). Effect of light colour temperature and intensity on the behaviour of male C57CL/6J mice. *Applied Animal Behaviour Science*, *184*, 135–140.  
<https://doi.org/10.1016/j.applanim.2016.08.005>
- Kuleskaya, N., & Voikar, V. (2014). Assessment of mouse anxiety-like behavior in the light–dark box and open-field arena: Role of equipment and procedure. *Physiology & Behavior*, *133*, 30–38. <https://doi.org/10.1016/j.physbeh.2014.05.006>
- Ma, W.-P., Cao, J., Tian, M., Cui, M.-H., Han, H.-L., Yang, Y.-X., & Xu, L. (2007). Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats. *Neuroscience Research*, *59*(2), 224–230.  
<https://doi.org/10.1016/j.neures.2007.06.1474>
- Martin-Arenas, F. J., & Pintado, C. O. (2019). Results of the Open Field Test at different light intensities in C57 mice. In A.J. Spink, E.L. van den Broek, L.W.S. Loijens, M. Woloszynowska-Fraser, & L.P.J.J. Noldus (Eds.), *Proceedings of Measuring Behavior*.  
<https://doi.org/10.13140/RG.2.2.29920.74247>
- Miller, C. K., Halbing, A. A., Patisaul, H. B., & Meitzen, J. (2021). Interactions of the estrous cycle, novelty, and light on female and male rat open field locomotor and anxiety-related behaviors. *Physiology & Behavior*, *228*, 113203.  
<https://doi.org/10.1016/j.physbeh.2020.113203>
- Moore, S. J., Deshpande, K., Stinnett, G. S., Seasholtz, A. F., & Murphy, G. G. (2013). Conversion of short-term to long-term memory in the novel object recognition paradigm.

*Neurobiology of Learning and Memory*, 105, 174–185.

<https://doi.org/10.1016/j.nlm.2013.06.014>

Morales-Delgado, N., Popović, N., De la Cruz-Sánchez, E., Caballero Bleda, M., & Popović, M. (2018). Time-of-Day and age impact on memory in elevated plus-maze test in rats.

*Frontiers in Behavioral Neuroscience*, 12, 304. <https://doi.org/10.3389/fnbeh.2018.00304>

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts.

*Learning & Memory*, 9(2), 49–57. <https://doi.org/10.1101/lm.41302>

Poulter, S. L., Kosaki, Y., Sanderson, D. J., & McGregor, A. (2020). Spontaneous object-location memory based on environmental geometry is impaired by both hippocampal and dorsolateral striatal lesions. *Brain and Neuroscience Advances*, 4, .

<https://doi.org/10.1177/2398212820972599>

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, 463(1–3), 3–33.

[https://doi.org/10.1016/S0014-2999\(03\)01272-X](https://doi.org/10.1016/S0014-2999(03)01272-X)

Rabat, A. (2007). Extra-auditory effects of noise in laboratory animals: The relationship between noise and sleep. *Journal of the American Association for Laboratory Animal Science*, 46(1), 35–41.

Ramos, A., Berton, O., Mormède, P., & Chaouloff, F. (1997). A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behavioural Brain Research*, 85(1), 57–69.

[https://doi.org/10.1016/S0166-4328\(96\)00164-7](https://doi.org/10.1016/S0166-4328(96)00164-7)

- Ramos, A., Kangerski, A. L., Basso, P. F., Da Silva Santos, J. E., Assreuy, J., Vendruscolo, L. F., & Takahashi, R. N. (2002). Evaluation of Lewis and SHR rat strains as a genetic model for the study of anxiety and pain. *Behavioural Brain Research*, *129*(1–2), 113–123. [https://doi.org/10.1016/S0166-4328\(01\)00337-0](https://doi.org/10.1016/S0166-4328(01)00337-0)
- Ramos, A., Mellerin, Y., Mormède, P., & Chaoulouff, F. (1998). A genetic and multifactorial analysis of anxiety-related behaviours in Lewis and SHR intercrosses. *Behavioural Brain Research*, *96*(1–2), 195–205. [https://doi.org/10.1016/S0166-4328\(98\)00023-0](https://doi.org/10.1016/S0166-4328(98)00023-0)
- Ramos, A., Pereira, E., Martins, G. C., Wehrmeister, T. D., & Izídio, G. S. (2008). Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. *Behavioural Brain Research*, *193*(2), 277–288. <https://doi.org/10.1016/j.bbr.2008.06.007>
- Rojas-Carvajal, M., Fornaguera, J., Mora-Gallegos, A., & Brenes, J. C. (2018). Testing experience and environmental enrichment potentiated open-field habituation and grooming behaviour in rats. *Animal Behaviour*, *137*, 225–235. <https://doi.org/10.1016/j.anbehav.2018.01.018>
- Saré, R. M., Lemons, A., & Smith, C. B. (2021). Behavior testing in rodents: Highlighting potential confounds affecting variability and reproducibility. *Brain Sciences*, *11*(4), 522. <https://doi.org/10.3390/brainsci11040522>
- Schmitt, U., & Hiemke, C. (1998). Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. *Pharmacology Biochemistry and Behavior*, *59*(4), 807–811. [https://doi.org/10.1016/S0091-3057\(97\)00502-9](https://doi.org/10.1016/S0091-3057(97)00502-9)

- Shanazz, K., Dixon-Melvin, R., Bunting, K. M., Nalloor, R., & Vazdarjanova, A. I. (2021). Light-dark open field (LDOF): A novel task for sensitive assessment of anxiety. *Journal of Neuroscience Methods*, *363*, 109325. <https://doi.org/10.1016/j.jneumeth.2021.109325>
- Shoji, H., & Miyakawa, T. (2021). Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: A challenge against conventional interpretation of the test. *Molecular Brain*, *14*(1), 34. <https://doi.org/10.1186/s13041-020-00721-2>
- Sidlo, J., Zaviacic, M., & Kvasnicka, P. (1995). Night and day differences in the food-intake of laboratory rats Wistar and Koletsky strains. *Bratislavské Lekárske Listy*, *96*(12), 655–657.
- Soler, J. E., Robison, A. J., Núñez, A. A., & Yan, L. (2018). Light modulates hippocampal function and spatial learning in a diurnal rodent species: A study using male Nile grass rat (*Arvicanthis niloticus*). *Hippocampus*, *28*(3), 189–200. <https://doi.org/10.1002/hipo.22822>
- Tsuda, M. C., Mahdi, S., Namchuk, A., Wu, T. J., & Lucki, I. (2020). Vendor differences in anxiety-like behaviors in female and male Sprague Dawley rats. *Physiology & Behavior*, *227*, 113131. <https://doi.org/10.1016/j.physbeh.2020.113131>
- Vierck, C. J., Acosta-Rua, A. J., Rossi, H. L., & Neubert, J. K. (2008). Sex differences in thermal pain sensitivity and sympathetic reactivity for two strains of rat. *The Journal of Pain*, *9*(8), 739–749. <https://doi.org/10.1016/j.jpain.2008.03.008>

Vogel-Ciernia, A., & Wood, M. A. (2014). Examining object location and object recognition memory in mice. *Current Protocols in Neuroscience*, 69(1).

<https://doi.org/10.1002/0471142301.ns0831s69>

Wahlsten, D., Bachmanov, A., Finn, D. A., & Crabbe, J. C. (2006). Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades.

*Proceedings of the National Academy of Sciences*, 103(44), 16364–16369.

<https://doi.org/10.1073/pnas.0605342103>