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SHORT COMMUNICATION

Constant light and single housing alter novelty-induced locomotor activity and sociability in female Swiss Webster mice

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Abstract

OBJECTIVE: Light exposure at night is known to produce behavioral aberrations in both human and animal models. One way to mimic light-at-night is through constant light exposure (LL), wherein animals are placed in an environment where a dark phase never occurs. Additionally, the type of housing condition for the rodents in experiments – grouped-housed vs singly-housed – can produce different behavioral responses, even in female mice. This study investigated whether LL produces alterations to emotionality and sociability, and whether group housing can alleviate some of those negative behavioral outcomes in female mice.

METHODS: Female Swiss Webster mice were placed into group or single housing conditions and either into a standard 12:12 light:dark cycle or LL. Novelty-induced (open-field, light-dark box) and circadian locomotor activity, sociability, and serum oxytocin were measured during the middle of the day.

RESULTS: LL and group-housing produced alterations to circadian home-cage activity and increases novelty-induced locomotor activity in the open-field and light-dark box. LL led to increased aggression in both group-housed and single-housed mice, while single-housed/LL mice showed reduced encounters towards the social mouse. Group-housed/LL mice exhibited increased interactions with the empty enclosure. Additionally, both LL and group-housing increased oxytocin levels.

CONCLUSIONS: The increase in oxytocin may be a contributing factor to why female mice exhibit increased aggression and other impaired social behaviors in LL. Socialization via group housing was ineffective in reducing the negative sociability seen in mice under LL. These results indicate that aberrant light exposure and circadian misalignment are correlated with impaired social behaviors and emotionality.

INTRODUCTION

One unfortunate here-to-stay part of modern life is exposure to light-at-night either through shift work or through exposure to building lights and streetlights. Brightening of the night-sky with human-made, artificial lighting at night can cause disruptions to the endogenous biological clock, which is evolutionarily conserved in all terrestrial animals, leading to adverse consequences. Multiple studies have shown in both humans and animal models that exposure to circadian disruption through light-at-night or continuous light (LL), can lead to abnormal behaviors, including, increased impulsivity, depression, and anxiety (Capri *et al.* 2019; Deane *et al.* 2021). The desynchrony between the environment and the endogenous biological clock provides one of the mechanisms to why disruptions to the lighting cycle or other chronobiological disruptions may lead to impaired emotionality and behavior (as reviewed by Hood & Amir, 2018; Deibel *et al.* 2020).

Nevertheless, research investigating the interactions between neurobehaviors and circadian rhythms usually employ singly housed mice, as measuring their circadian locomotor activity or behavioral/physiological phase (i.e., a specific point in a cycle) can be easily measured. However, previous work with mice has shown that significant differences in behavioral outcomes can occur depending on whether they are group housed and allowed to socialize or housed alone (Voikar *et al.* 2005). Group housing has been shown in both male and female rats (Oliveira *et al.* 2021) and mice (Voikar *et al.* 2005; Liu *et al.* 2013) to alleviate stress responses and reduce aggression and anxiety-like behaviors. While numerous studies have linked sleep deprivation to abnormal social behavior (Hood & Amir, 2018; Deibel *et al.* 2020), very few studies have investigated the impact of altered circadian photoperiods or circadian disruption, independent of sleep restriction, on the sociability of rodent models. As such, the purpose of this study was to determine whether group housing can alleviate some of the negative behavioral consequences of LL exposure in female mice.

MATERIAL AND METHODS

Animals

This study had the approval of Bridgewater State University's Institutional Animal Care and Use Committee. Thirty-two female Swiss Webster mice (024) aged 8-weeks were purchased from Charles River Laboratories (Shrewsbury MA, USA). Mice were either placed into a group housing setting (3 per cage) or single housing condition into cages that were able to monitor their circadian locomotor activity via an infra-red beam (Starrlife Sciences, Oakmont, PA, USA). After one week of acclimation to their new environment, mice were then placed either into a standard 12:12 light:dark cycle (LD – 0600-1800 lights on) or constant light (LL – 24 h lights on, approximately 100 lux), so that there were four groups: 1) Group-housed/LD (n=9), 2) Group-housed/LL (n=9), 3) Single-housed/LD (n=7), and 4)

Tab. 1. Open-field and Light-Dark Box Assays. Different letters (a,b,c) indicates significantly different from each other, at $p \leq 0.050$.

Open Field						**L-D Box (Overall)**	
Housing	**Cycle**	**Distance (cm) (LD < LL)**	**Velocity (cm^2/sec) (LD < LL)**	**Rears**	**Center Zone Time (min)**	**Dark Zone Time (min)**	**Transitions**
Group	LD	1266.09 ± 114.42[a]	3.29 ± 0.14[a]	53.33 ± 3.56[a]	1.24 ± 0.19[a]	4.04 ± 0.32	24.56 ± 2.35[a]
Group	LL	1374.01 ± 94.52[a]	3.39 ± 0.16[a]	59.75 ± 5.63[b]	1.07 ± 0.16[a]	3.52 ± 0.34	29.11 ± 3.00[b]
Single	LD	1463.54 ± 117.93[b]	4.02 ± 0.24[b]	43.36 ± 11.03[a]	0.80 ± 0.10[b]	3.95 ± 0.48	27.57 ± 3.43[a]
Single	LL	1764.53 ± 183.97[b]	4.36 ± 0.27[b]	63.14 ± 4.70[b]	0.88 ± 0.082[b]	3.93 ± 0.42	40.29 ± 6.82[b]

L-D Box Locomotor Activity (Dark Zone)					**L-D Box Locomotor Activity (Light Zone)**		
Housing	**Cycle**	**Dark Zone Distance (cm)**	**Dark Zone Velocity (cm^2/sec)**	**Dark Zone Rears**	**Light Zone Distance (cm)**	**Light Zone Velocity (cm^2/sec)**	**Light Zone Rears**
Group	LD	485.11 ± 41.06[a]	2.67 ± 0.13[a]	15.11 ± 1.16[a]	667.49 ± 31.56[a]	2.70 ± 0.10[a]	32.00 ± 3.30[a]
Group	LL	529.35 ± 43.09[a]	3.36 ± 0.18[b]	17.00 ± 1.81[b]	818.06 ± 31.83[b]	3.12 ± 0.11[b]	34.13 ± 2.40[b]
Single	LD	600.74 ± 66.89[b]	3.21 ± 0.24[b]	15.33 ± 3.44[a]	738.16 ± 83.67[a]	3.03 ± 0.17[a]	27.83 ± 4.61[a]
Single	LL	700.81 ± 76.26[b]	3.22 ± 0.094[b]	26.21 ± 5.46[b]	897.96 ± 133.08[b]	3.33 ± 0.23[b]	39.21 ± 3.79[b]

Single-housed/LL (n=7). Mice were given standard chow (LabDiet 5001) and water *ad libitum*. All of the following assays listed below were conducted during the middle of each mouse's inactive time (approximately Zeitgeber Time (ZT) 6 for LD animals and Circadian Time (CT) 6 for LL animals) and in the light to form the basis of comparison.

Behavioral Assays

Each mouse was assayed in the open field and light-box tests after 8 weeks and 9 weeks (respectively) of exposure to their respective lighting conditions as previously described (Capri *et al.* 2019). The open-field and the locomotor activity parameters of the light-dark box (transitions and open-field-type measurements) were used to assess novelty-induced activity and impulsivity/darting behaviors, while dark-zone time within the light-dark box was used as a measure of anxiety-like behavior.

After 10 weeks of exposure to their lighting condition, each mouse was tested in a novel sociability assay using the SmartCage System (Afasci Inc. Redwood City, CA, USA). Mice were placed into the same apparatus as the open field, but with two smaller enclosures placed on opposite ends of the open field. These enclosures had metal mesh surrounding it to allow for interactions between the animals but not complete touching. At first mice were able to acclimate to the empty cage for 10 minutes. Afterwards, a grouped-housed (in LD) female C57BL/6J mouse of approximately 2 months old was placed into the right (to the experimenter) smaller mesh cage and served as the social partner for the subject mouse. Mice were observed for 10 minutes for aggressive behaviors (e.g., attempting to bite and hit mouse through mesh enclosure, tail rattling, defensive postures) and non-aggressive behaviors (e.g., sniffing, rearing with paws on the enclosure, gentle touching of mesh enclosure or mouse) toward the social partner and the empty cage that lasted for at least one second was recorded manually. Additionally, the amount of time spent in the zone with the empty cage and the social partner but not interacting with either cage was also recorded.

Blood Oxytocin

One week after the sociability assay, whole blood was collected from each mouse at ZT 6 or CT 6, allowed to clot at room temperature for at least 2 hours, and centrifuged at 2000g for 20 minutes at 4°C to obtain serum. Serum was then tested for oxytocin levels using Oxytocin ELISAs (#MBS776702, MyBioSource, San Diego, CA, USA).

Statistical Analyses

The circadian period and phase of each animal were calculated using Clocklab's (Actimetrics, Wilmette, IL, USA) automated chi-square periodogram and automatic onset and predict functions. Two-way ANOVAs with Tukey post-hoc tests were conducted for all dependent variables in this study.

RESULTS

Circadian and Novelty-induced Locomotor Activity

The means and SEM of the circadian, open field, and light-dark box parameters are summarized in Table 1. For the open field, group housing led to decreases in distance traveled ($p = 0.020$) and velocity ($p = 0.001$), while producing increases in center zone time ($p = 0.035$). LL led to increases in rearing behavior ($p = 0.042$), but none of the other parameters. For the light-dark box, LL led to increases in the number of transitions between the two zones ($p = 0.027$), but no differences were found for dark zone time ($p = 0.63$). In the dark zone, group housing decreased the distance traveled ($p = 0.012$). An interaction was found for dark zone velocity ($p = 0.044$); while group/LD animals exhibited reduced velocity compared to group/LL animals ($p = 0.014$), no differences were found between LD and LL for single housed animals ($p = 0.99$). In the light zone, LL produced increases to distance ($p = 0.030$) and velocity ($p = 0.016$), while housing conditions had no effects. Rearing was increased for mice in LL in both the light ($p = 0.050$) and dark zones ($p = 0.042$).

Sociability

LL led to increases in aggressive encounters with the novel mouse ($p < 0.001$), but group housing had no effects (Figure 1a). Interactions were uncovered for non-aggressive encounters ($p = 0.049$) and encounters with the empty cage ($p = 0.050$). Whereas no differences were found between group housed mice in LD vs. LL ($p = 0.93$), single/LD mice exhibited increased non-aggressive interactions with a novel mouse compared to single/LL ($p = 0.014$) (Figure 1b). Additionally, group/LL mice had increased encounters with the empty enclosure compared to all other groups ($p = 0.027$) (Figure 1c). No differences were found in the time spent in either zone containing the novel mouse (Figure 1d) or the empty enclosure (Figure 1e). Oxytocin was increased in mice experiencing group housing ($p = 0.002$) and LL ($p = 0.010$) (Figure 1f).

DISCUSSION

In this study, female Swiss Webster mice experienced impaired sociability when exposed to LL, manifested in reduced non-aggressive interactions in single-caged mice, increased interactions with the empty cage in group-housed mice, and increased aggression in all housing conditions. A late chronotype and longer days during summer are associated with an increased likelihood of aggressive behaviors compared to morning type individuals and shorter days in humans (Hood & Amir, 2018; Deibel *et al.* 2020), so the period lengthening seen in these animals placed in LL may

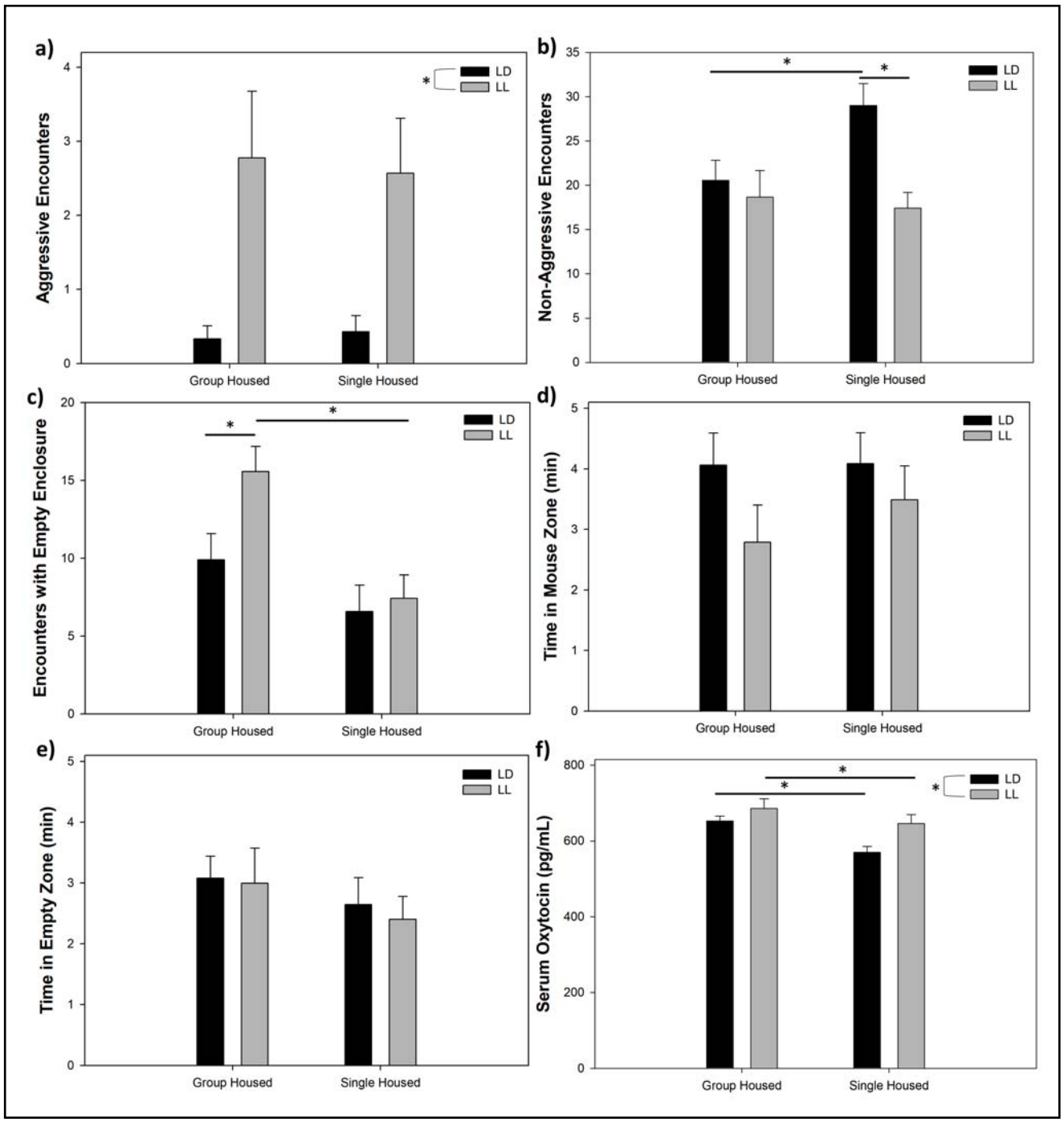

Fig. 1. Sociability and Blood Oxytocin Levels. a) Aggressive encounters; b) Non-aggressive encounters; c) Interactions with the empty enclosure; d) Time spent in mouse zone; e) Time spent in empty zone; f) Serum oxytocin levels. *: significantly different from each other at $p \leq 0.050$.

be a contributing factor for the increased aggression found in this study. Evening types also report poorer sleep outcomes, which is also associated with increased impulsivity and aggression (Hood & Amir, 2018). Additionally, sundowning in Alzheimer's patients, is associated with increased aggression later in the day, may also be related to this phenomenon. Although there are several mechanisms leading to sundowning, circadian desynchrony and period lengthening may be a contributing factor to this effect as circadian disruption can exacerbate sundowning symptoms (Deibel *et al.* 2020). Other work also has demonstrated that mice genetically modified to lack genes that regulate the molecular clock loop, such as clock, or exhibit altered transmission of circadian rhythm related neurotransmitters, such as serotonin, GABA, and dopamine, also display increased aggressive behaviors towards a novel individual and increased impulsivity (Coque *et al.* 2011; Todd *et al.* 2018). Other forms of circadian disruption, such as jet-lag,

shift-work, or abnormal daylengths are also associated with negative social behavior (Hood & Amir, 2018; Deibel *et al.* 2020). Lastly, a recent study has shown that there is circadian regulation of aggression through the circadian timing system in constant conditions, even without the presence of external lighting cues (Todd *et al.* 2018). The current study and previous work all suggest that the circadian clock plays a role in the regulation of social behavior and that circadian desynchrony that promotes poorer sleep and later chronotypes is correlated with aggressive and antisocial behaviors.

While it is expected that mice experiencing group housing conditions would express increased oxytocin due to increased social interactions on a daily basis (as seen in other studies such as Liu *et al.* 2013), it was somewhat surprising that increased oxytocin along with increased aggression was found in female mice experiencing LL, regardless of housing condition. Recent work, however, has linked aggression to increased levels of oxytocin in female rodent models, where endogenous oxytocin promotes aggression through activation of the ventral lateral septum (Oliveira *et al.* 2021). Additionally, a prior study reported that serum oxytocin and oxytocin immunopositive cells within the paraventricular nucleus of the hypothalamus are elevated in long days (16 h of light per day) compared to short days (8 h of light) (Trainor *et al.* 2010). The increase in oxytocin due to increased light exposure as well as the link between endogenous oxytocin and aggression in female rodents may be a reason to why prior socialization through group housing did not alleviate the negative social behaviors of mice housed in LL when presented with a novel mouse.

This study also adds to the body of evidence that exposure to LL increases acute novelty-induced locomotor activity in mice, manifested in increased distance traveled, rearing, velocity, and transitions between light and dark zones in the open-field and light-dark box. Other studies using a variety of strains and sexes of mice have also found that LL increases these locomotor behaviors, which may be interpreted as a sign of impulsivity (Capri *et al.* 2019; Deane *et al.* 2021). Again, group housing for female mice was mostly unsuccessful in alleviating the altered emotionality (increased darting-like behavior as a measure of impulsivity) caused LL. One possibility for the behavioral changes seen in this study is that LL may be causing increased HPA activation and producing a stress response. Still, center-zone time within the open-field was increased in group-housed animals, regardless of lighting conditions. This result may imply a decrease in HPA-axis activation that is associated with group housing of rodents when placed into an open-field assay as seen in a previous study (Liu *et al.* 2013). Oxytocin administration is associated with reduced stress levels in animal models (Peters *et al.* 2014; Wang *et al.* 2018) and oxytocin knockouts exhibit increased susceptibility to stress and elevated corticosterone levels (Amico *et al.* 2004). On the other hand, while oxytocin administration is associated with reduced stress responses, stressors appear to increase brain levels of oxytocin within the paraventricular nucleus of the hypothalamus particularly in female rodents (Babygirija *et al.* 2012; Steinman *et al.* 2016). Interestingly, a study investigating the effects of chronic stress on plasma oxytocin levels reported that chronic stress elevated plasma oxytocin during the day but had no effects during the night (Dubovicky *et al.* 2007). This result implies that the chronic stress of exposure to LL could increase blood oxytocin levels without improving the impaired sociability and emotionality found in both the group and single-housed mice in this study, since the mice were only tested during the subjective day and in the light. It is worth noting, however, that the HPA axis, nor brain oxytocin levels, were not explicitly tested in this study, so future work connecting behavior (including sociability) and stress due to circadian disruption is needed.

Still, the behavioral assays and oxytocin measurements were conducted at a single timepoint along their circadian cycle (middle of the day) and that phase of the grouped-housed mice was estimated using a single circadian locomotor activity monitor for the three animals. As such, the calculated phase of the animals within the same cage may have been slightly different than if they were housed alone, where we know the activity of each individual animal. Additionally, the results may be slightly different if measured at a different time, even if in the light, such as during the beginning or the end of the light period. Nevertheless, this study supports previous work that LL can produce altered behaviors in female mice, similar to what is found male mice, even during the light phase. Additionally, this study also notes that the type of housing condition (single vs. group) can modulate behaviors in female rodents, independent of lighting cycle.

In summary, this study provides some of the first evidence that aberrant photoperiods and alterations to the circadian rhythm can produce abnormal social behaviors in female mice. Female Swiss Webster mice in LL displayed significant alterations in behavioral outcomes and oxytocin levels compared to animals held in a standard LD cycle. Prior socialization through group housing of the animals was not able to ameliorate all of the negative social effects of LL, particularly aggression, towards a novel mouse and was also ineffective in mediating the impulsivity effects of LL in the open-field and light-dark box assays. Further work is still needed to elucidate the mechanisms behind how altered circadian rhythms can lead to abnormal locomotor, impulsive, social, and aggressive behaviors.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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