

# Behavioral Adaptations of Female Mice on the International Space Station

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## Introduction

In 2014, 20 female mice (10 used by NASA) were flown to the International Space Station (ISS) aboard SpaceX-4 as part of a 37-day validation mission designed to assess the performance of the Rodent Research Hardware System as a suitable housing environment for mice during long-duration spaceflight. This was the first Rodent Research mission (RR-1), part of an ongoing program of research with the aim of better understanding the influence of microgravity conditions on mammalian physiology and well-being, with the ultimate goal of identifying potentially harmful effects of the space environment and thereby facilitating Exploration Class missions and the eventual human colonization of space. We previously found that the RR-1 flight mice exhibited on orbit the same forms and levels of species-typical behavior as identically-housed ground controls, and intact circadian timing assessed late during the flight (unpublished data). As compared to controls, spaceflight mice exhibited significantly greater overall activity, including a socially organized circling or 'race-tracking' behavior that emerged within 2 weeks of launch, and became the predominant behavioral activity of spaceflight mice during the dark cycle phase. The present study is the first attempt to analyze social interactions and possible anxiety-related behaviors in mice to assess how well they adapt to potentially stressful long-duration habitation of space. Thigmotaxis, or the tendency to remain in close proximity to walls and corners, is a common measure of anxiety in rodents (e.g. Lamprea et al., 2008). The expectation motivating the measurement of thigmotaxis in this study was that the novel experience of microgravity may produce anxiety in the mice, and if so, an effect of habituation to that anxiogenic stimulus may be apparent within the first few days of spaceflight. That is, if the mice are initially anxious and therefore more prone to stay within corners formed by two or more adjoining walls, then we should see a clear and rapid decrease in thigmotaxis as they subsequently become more comfortable within their new environment.

## Methods

Making use of video acquired daily for 33 days from within the Rodent Habitat, a selection of behavioral measures were recorded in an attempt to assess the acclimation of the mice to the microgravity environment. The subjects are 16-week-old female mice ( $N = 10$  in-flight *C57BL/6*;  $N = 10$  ground control *C57BL/6*), but because tracking them as individuals across videos is impossible, frequencies and durations of their behaviors were pooled into single values for each flight day. The left and right housing areas of the Rodent Habitat (5 mice inhabiting each) are independent environments and were treated as such in our statistical analysis. BORIS (Friard & Gamba 2016), a free, open-source event-logging program, was used to record frequencies and durations of the behavioral categories, and R was used for data processing and analysis.

The categories of behavior under analysis were: Allo-Grooming, Aggression, Proximity, and five categories representing thigmotaxis, an index of anxiety. However, because it was observed so rarely, allo-grooming was ultimately excluded from the later analyses. Behaviors are expressed as either average occurrences per minute by flight day, or as total duration normalized to video segment duration.

## Operational Definitions of Key Behaviors

**Sniff:** mouse conspicuously directs its head toward another mouse and appears to sniff her, i.e. snout is very close to and oriented toward any part of the other mouse.

**Aggression:** any behavior that is unambiguously aggressive, i.e. one mouse nips another, pulls out another's fur, pulls its tail, etc.

**Proximity:** one mouse moves to within very close proximity of another and remains in proximity for appreciable amount of time, e.g. is clearly not just passing by. Joining is considered to have stopped when either the mouse/mice being 'joined' moves out of proximity to the 'joiner', or the 'joiner' orients away from the mouse/mice and begins to move away. This was intended to be a general measure of interaction among the mice and time spent in close proximity with one another.

**Thigmotaxis:** whether the mouse was near a wall or corner (thigmotactic) or in the 'open-field' (non-thigmotactic), and whether the mouse was stationary or ambulating while in that location, collectively forming five categories of 'thigmotactic state'.

## Results

As seen in Figure 1, the hypothesized linear trend of a rapid reduction in thigmotactic behavior across the first few days of exposure to microgravity aboard the ISS failed to appear in either of the two environments, and the margin of error (MOE) for the correlations between flight day and duration of thigmotaxis was unacceptably high for every condition described in Figure 1 (MOE on 95% CI of  $r > 0.9$ ), indicating no evidence of a linear association. A one-way analysis of variance, comparing normalized thigmotaxis durations across all housing conditions, indicated statistically significant differences ( $F(3, 12) = 7.758, p = 0.0038$ ). A post-hoc Tukey's HSD test illustrates that thigmotactic duration differed significantly only between the right ISS and left ISS environments ( $p < 0.05$ ) and between the right ISS and left KSC environments ( $p < 0.05$ ), and approached significance between the right ISS and right KSC environments ( $p = 0.055$ ). One additional noteworthy result was the finding of no aggressive interactions among the in-flight mice, in contrast to the ground controls housed at Kennedy Space Center.

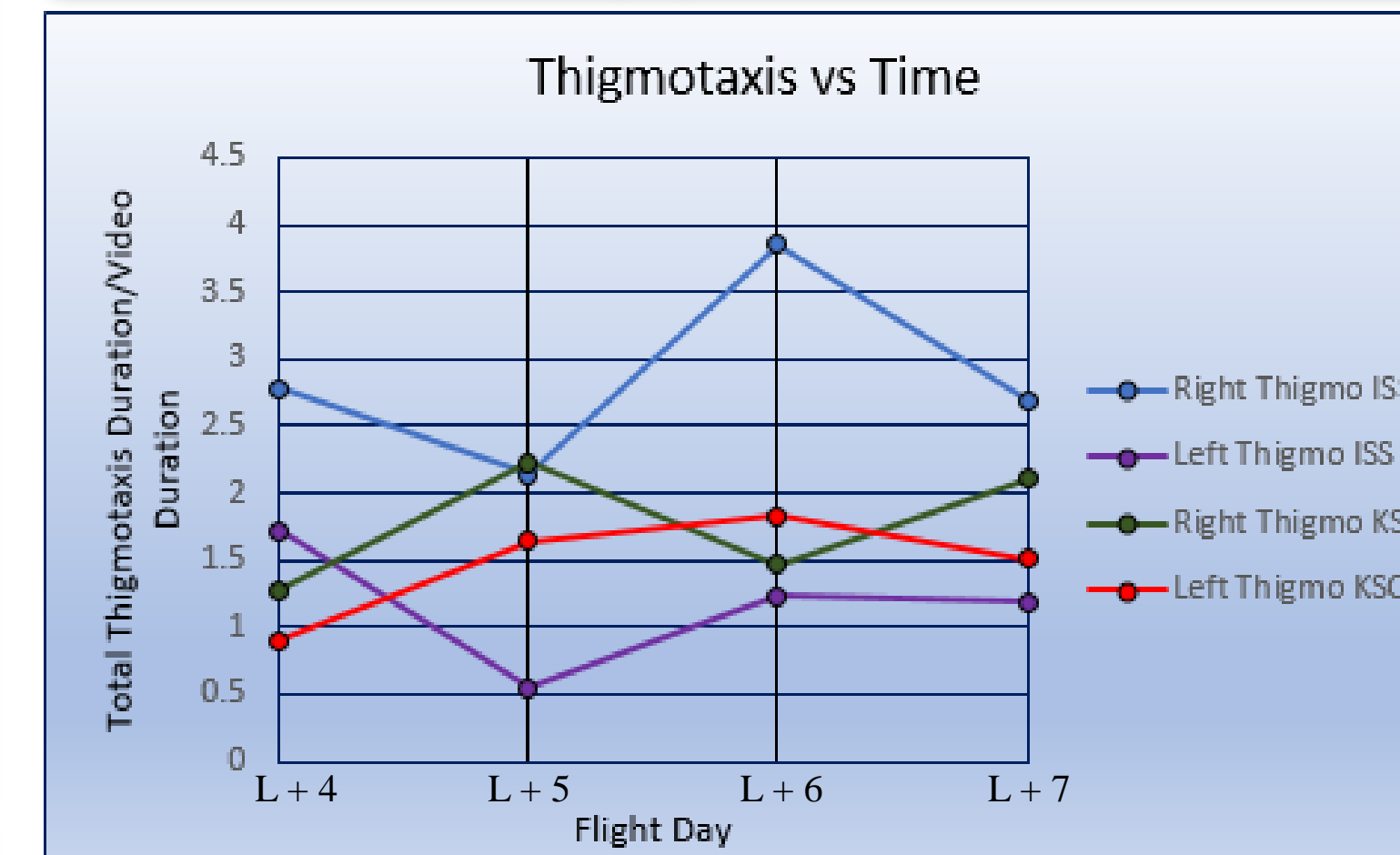


Figure 1. Total, combined durations of thigmotactic states normalized to the duration of dark cycle video during which mice were visible (sum of total left and right filter time). "Right" and "Left" refer to the two sides of the habitat, treated as independent environments

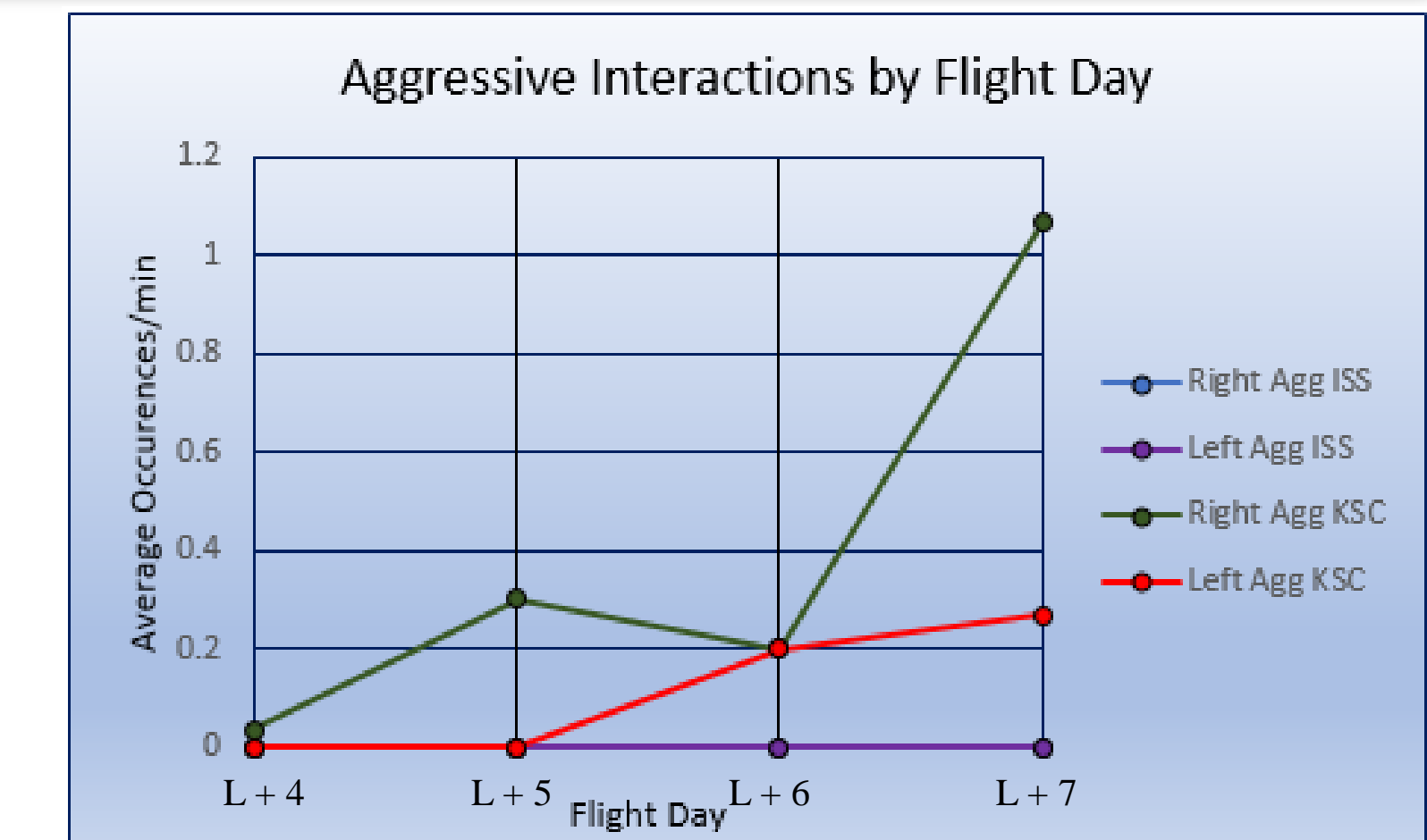
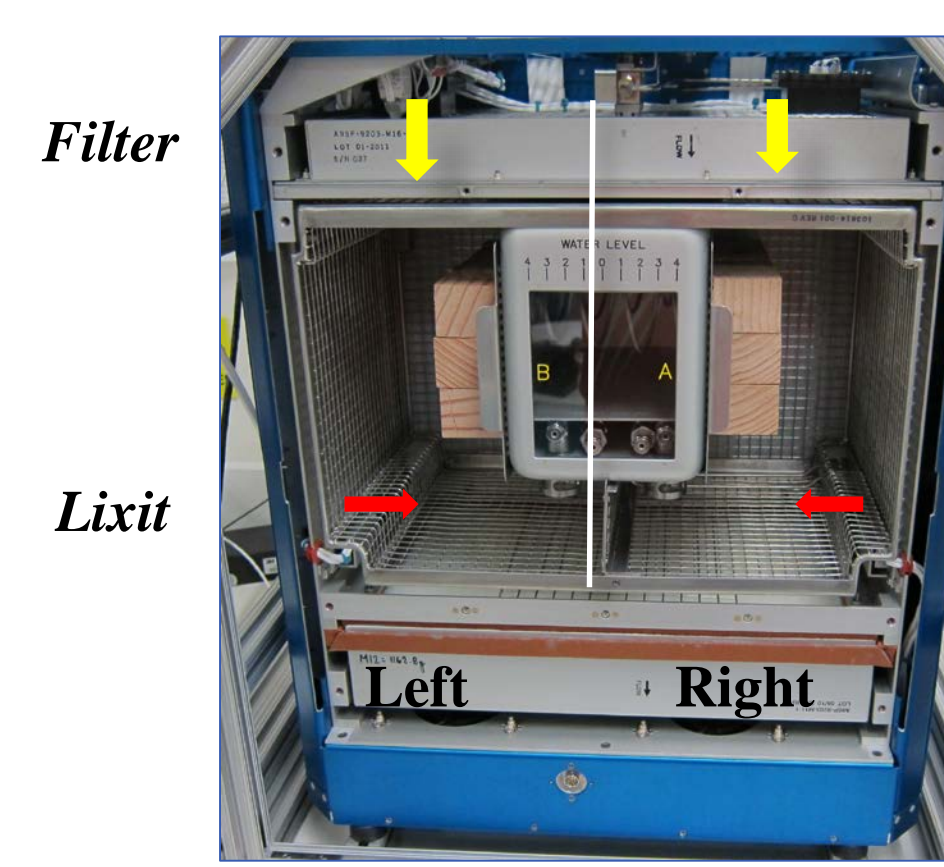


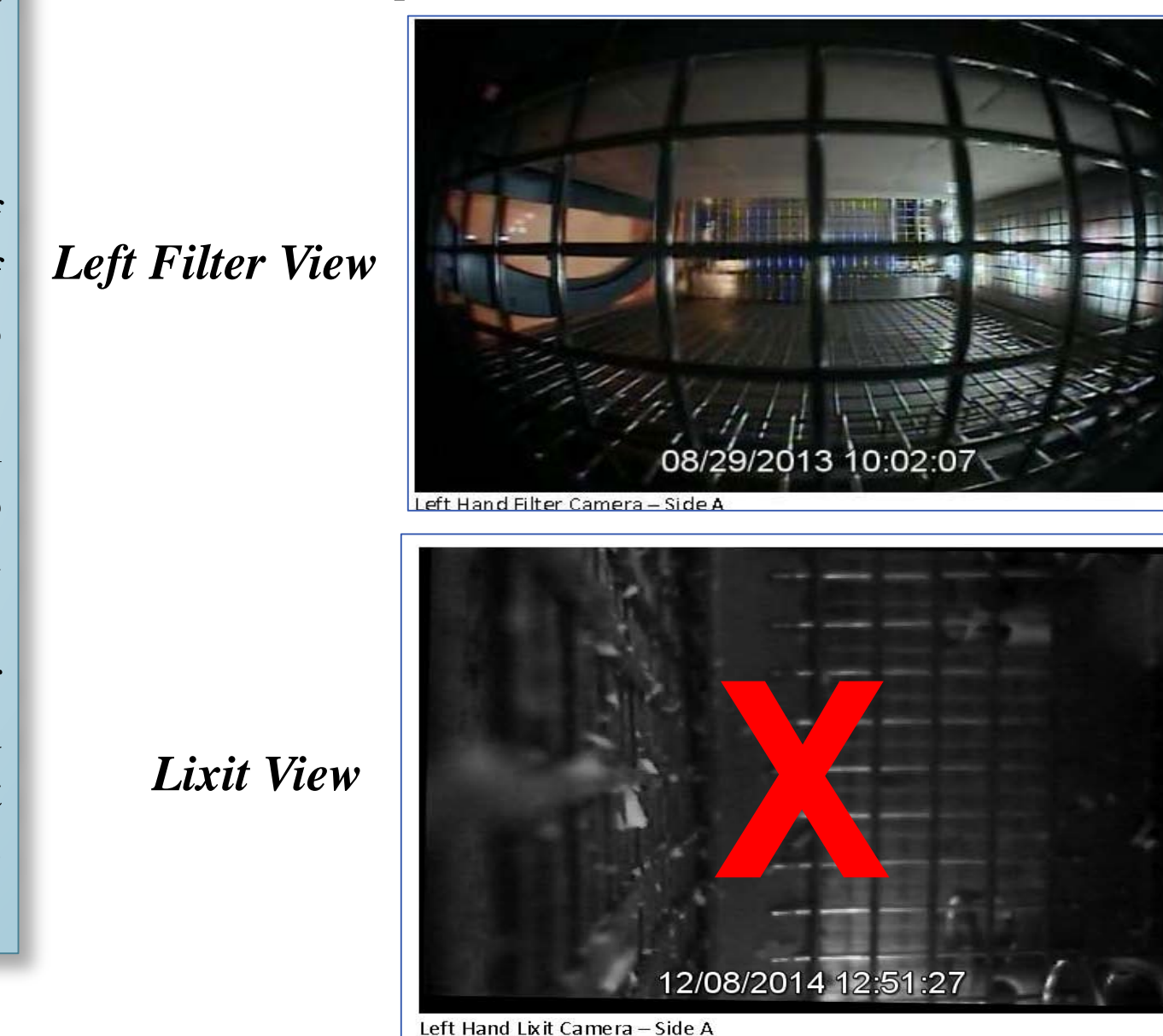
Figure 2. Instances of "Proximity" normalized to total left and right filter time, plotted against day aboard the ISS. Error bars represent the standard error of the mean.



Rodent Habitat (RH)



Dual Housing Areas with Designated Camera Positions



Left Filter View

Lixit View

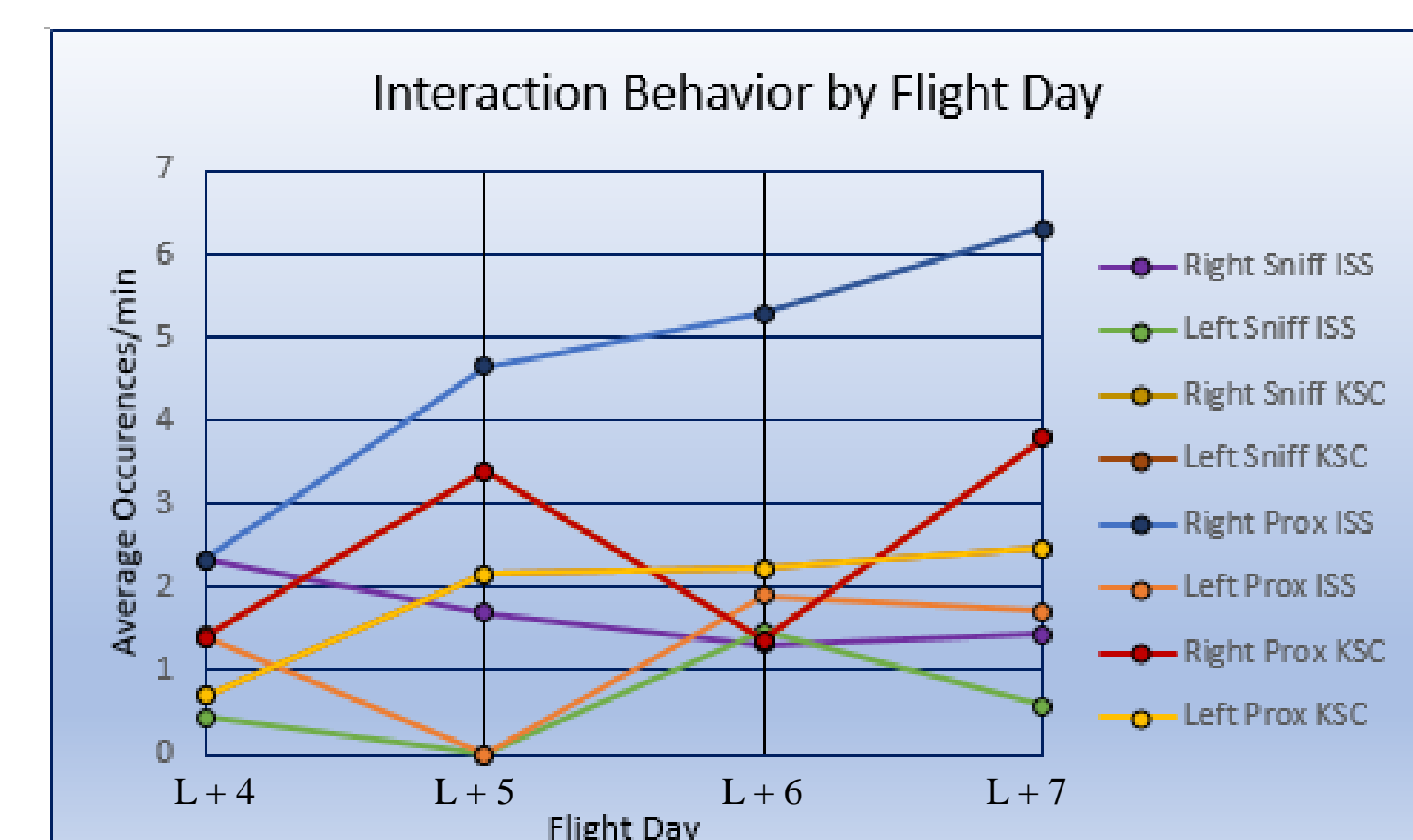


Figure 3. Instances of one mouse sniffing or moving into close proximity to another, normalized by dividing by the video duration in seconds, then multiplied by 60 to yield a value in terms of occurrences per minute by flight day.

## References and Acknowledgements

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- Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7(11), 1325-1330.

NASA Office of Education, NASA Space Biology Program, NASA Ames Biosciences Research Division, and members of the NASA Reproduction and Development Lab

## Summary and Conclusions

The results of the ANOVA and post-hoc Tukey's test indicated that only one environment, the right housing area of the in-flight Rodent Habitat, was significantly different from the others in the duration of thigmotactic behavior observed within it. These results are consistent with our past finding that have demonstrated greater activity in the right housed mice aboard the ISS. The observation of a statistically significant difference in thigmotaxis between only one housing area of the in-flight habitat and the ground controls, as well as a significant difference in the same variable across the two in-flight housing, in combination with our past findings suggest that the space-flight environment alone could not account for the differences in anxiety in the mice. However, the right-sided phenomenon was not limited to just thigmotaxis, our results also showed that the GC right-sided habitat had greater instances of aggression than the left and that both GC habitats had much greater occurrences of aggression than aboard the ISS. The influence of the habitat on mouse behavior is of great importance for the success of future missions and further research is needed. However, our ability to interpret these findings are limited based on the novel adaption of the definition of "thigmotaxis," the small sample size and the limited duration of usable video.