A novel BOX maze learning paradigm measures sleep-induced cognitive impairment

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2019

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Program Authorized to Offer Degree:

Comparative Medicine

University of Washington

Abstract

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Sleep deprivation-induced cognitive impairment is a major health concern in developed countries and is an age-related risk factor for neurological conditions such as dementia associated with Alzheimer's disease. There is an urgent need to develop ways of preventing the adverse neurological effects of sleep deprivation, but current preclinical cognitive assessments are highly time intensive with constant monitoring and at times generating data difficult to translate clinically. In this regard, a single day assay that focused just on assessing learning behavior in a time-effective and relatable manner would be of value. This report describes validation of the BOX maze as a behavioral paradigm for learning impairment in short-term sleep deprivation studies in mice. C57BL6 and C57BL/6xBALBc F1 (CB6F1) mice of both genders and varying ages were sleep deprived for 4 hours a day for 4 days, and then tested with the BOX maze. In some experiments, mice were treated with rapamycin daily during the 4 days of sleep deprivation. Sleep deprived mice showed learning impairment in an age, gender and strain dependent manner. The data provide evidence that the BOX maze is a behavioral paradigm that can be used to test short term sleep-deprivation induced learning impairment in young, middle,

and older aged C57BL/6 and CB6F1 mice of both genders. B6 female mice, 22 months of age, treated with rapamycin showed significant improvement in learning time suggesting the BOX maze could be a useful behavioral assessment in sleep deprivation drug studies. In conclusion, the BOX maze could be used as an initial cognitive screening assay, or to complement other commonly used mouse behavioral assays.

Keywords: Box Maze, Sleep Deprivation, Learning Impairment, Aging, Rapamycin

Dedication

This work is dedicated to the endless love and support of my dad Ashis, mom Boni, brothers Kingshuk and Kushal, sister Krishty and girlfriend Stevie.

Introduction

Sleep deprivation is a major health concern in developed countries and is associated with increasing age (1,28,29). Normal aging produces sleep disturbances including sleep fragmentation and sleep loss in humans (6,7). Abnormal aging causes an acceleration in the aging process and can promote age related health issues such as cancer, dementia, and heart disease. Sleep deprivation has an inflammatory reaction in systemic tissues and is known to cause exhaustion and impaired learning ability. The Center for Disease Control estimates that more than 35% of adults in the United States are affected by partial sleep deprivation (8,10,11). This partial sleep deprivation is defined as less than seven hours of sleep, though this changes with increasing age (34,35). The effects of sleep deprivation disturb the sleep cycle (12) and cause systemic repercussions such as poor physical recovery, impaired immunity, and memory and learning deficits (13,14,15). Often, individuals are at risk for falling asleep during tasks the following day (16). It has been shown that sleep deprivation impairs remote memory in aged mice, with changes in gene expression in the hippocampus (17). Sleep deprivation has detrimental effects on glucose metabolism in peripheral organs (18). In a chronic sleep deprivation experiment, aged animals became hyperglycemic and failed to maintain appropriate plasma insulin concentrations suggesting glucose dysregulation (19). A short-term sleep deprivation assay would be a useful tool to study sleep deprivation-induced cognitive impairments.

Mazes have been used extensively to test for behavioral effects in mice (1), with one of the purposes designed to assess different aspects of cognition. Cognition is defined as any type of

mental work including, learning, memory, working memory, spatial recognition, and new object recognition (2,3). Learning impairment falls under the larger umbrella of cognitive impairment and is defined by a deficit in the process of acquiring knowledge (NIH, NCI website). Long term memory and learning can be assessed by the radial water tread maze (5). There is a training phase which is highly time intensive. Many mazes exist which require mice to solve problems. They often have a memory and learning components (6). This is seen with mazes like the high wire walking maze and other raised platform mazes, which require the mouse to walk across a beam or balance on a platform. Some mazes have a water component as an adverse motivational stimulus. The Morris water maze and water tread maze are examples, which combine learning with memory over multiple days (4). The T-maze and Y-maze are fast but measure spontaneous and forced decision making. A learning assay with time-efficient readouts and conclusions could be highly impactful in research situations and allow for combining multiple assessments such as needed for sleep deprivation studies.

This report describes the validation of a learning paradigm, designated as the BOX maze, that could be used as a readout for short term sleep deprivation studies in aging mice with the ability to determine response to various medications which may rescue the cognitive dysfunction caused by lack of quality sleep. Data are presented showing the ability of the paradigm to distinguish sleep deprived learning impairment in an age, strain, gender and rapamycin drug treatment manner. Additional validation was established by showing the BOX maze aligned with the Y maze, a spontaneous decision-making assay.

Materials and Methods

Mice and Care

Mouse were housed in a standard 12:12 cycle. C57BL/6 (B6) mice provided by the National institutes of Aging. C57BL/6 x BALBc (F1) were provided by Jackson Laboratory. Two strains of mice were used because they are known to have a difference in baseline behavior. B6 mice are known to be more docile than F1 mice. It was important to be able to characterize whether or not this baseline behavior altered the performance of the BOX maze. Ages used were 8, 16, and 22 months. Monitoring for all mice on the study under 18 months of age: Mice were monitored at least once per week, but more frequently (daily) if mice began to show signs of poor health (24). This was determined in consultation with veterinary staff on a case by case basis. Mice were also weighed once per week. Mice were monitored for behavioral and clinical signs of poor health and were euthanized by CO_2 followed by cervical dislocation when they occurred. Mice were housed in groups of no more than five. They were provided with bedding, standard water and chow from the animal facility. No special dietary constraints were placed. Cages were changed every other week and experiments were scheduled to not coincide with cage changes. All procedures were performed as part of an approved scientific protocol in accordance with the University of Washington Institutional Animal Care and Use Committee (IACUC).

Sleep Deprivation

This study focused on partial sleep deprivation so a time range of 4 to 6 hours of sleep deprivation was selected in contrast to more long-term studies reported in the literature (31,32). Mice were handled for 7 consecutive days before beginning sleep deprivation so handling limited influence on behavior. Cage tapping was the primary method of sleep deprivation. Cage removal and replacement of the cage within the rack was a secondary method for mice which struggled to stay awake (26,27,30). Multiple intervals of sleep deprivation were tested and ultimately a 10 to 15-minute period between sleep disturbance was used (33). Based on a 12:12 dark/light cycle, the animals were sleep deprived starting 3 hours after lights came on. A total of four hours was the duration of sleep deprivation. The timeline of sleep deprivation was lengthened from one day to four days following the early results which did not disturb mice enough to behave differently in the BOX maze.

Learning assessment.

The BOX maze was constructed from a rectangular clear hard plastic box. Each side had 2-4-centimeter (cm) holes cut, and PVC caps were placed on 7 of them. One hole had a tube leading to an escape cage. The holes were cut and centered 3cm from the bottom of the cage (Figure 1). There was a hanging light of 800 lumens above the maze and the room lights were off. Each trial was preceded by a wipe down of the maze with 70% ethanol. This was to ensure the mouse could not follow its own odor back to the escape cage. Once the ethanol evaporated the mouse was given 120 seconds to find the escape hole. A completion of the maze was defined as all four paws of the mouse being in the escape hole. This endpoint marker was because the time for mice to reach the escape cage varied between trials and mice. If the mouse was unable to find the

escape hole it was gently picked up by the tail and shown the escape hole. The mouse was kept in the escape cage for about two minutes before running another trial. There were 4 trials in total (43,44). The BOX maze paradigm is a single day assay which takes roughly 15 minutes per mouse.

The Y maze is a commonly used tool to measure spontaneous reactions (9). The expectation is that an undisturbed mouse would be more likely to visit novel environments when compared to stressed mice. The Y maze uses three arms which are spaced apart evenly. Each arm is 12 inches in length. A mouse is placed in the end of one arm and given five minutes to wander the maze freely. Each arm that is visited is recorded and the final data is analyzed for novel arms visited compared to previously visited arms. When looking at the data in groups of three if the first and third arm visited match, then that third arm is considered a revisit.

Rapamycin treatment

Rapamycin, sourced from LC Laboratories, was prepared with dimethyl sulfoxide (DMSO) to make a stock concentration of 100mg/mL and then mixed into a solution of 5% Polyethylene Glycol, 5% Tween 80, and 90% Sterile H2O (45). Injection dose was 8 mg/kg. Average body weights of mice over the course of two weeks was taken to determine dose of 200 µg. Sham injections followed the same procedure without rapamycin component. Intraperitoneal injection method was used and approved by IACUC. Mice were handled for one week prior to starting injections. Injections were performed for a total of seven consecutive days. For mice which were part of the sleep deprived study, sleep deprivation began on the fourth day of injections. Mice in

this treatment trial ware separated into four categories each with 10 mice for a total of 40 mice (sleep deprived vehicle treated, non-sleep deprived vehicle treated, sleep deprived rapamycin treated, non-sleep deprived rapamycin treated).

Data Analysis

Data analysis was performed in Graphpad Prism. Graphs were created using Prism software after repeated measures 2-way ANOVA with post hoc analysis.

Results

The Box maze learning paradigm distinguished between sleep deprived and non-sleep deprived mice.

B6 male mice 8 months of age showed a pattern of learning across four separate trials when compared with sleep deprived mice (Figure 2A). Two-way ANOVA test suggested that there was a significant difference between sleep deprived and non-sleep deprived mice ($p<0.05$). The test also suggested that there was a significant difference for two or more trials. T-test supports this claim showing significance in 8-month male B6 mice in trial 2 and 3. ANOVA analysis of the data also showed a significant difference between the performance pattern of NSD and SD mice. This follows the visualization of the graph on Figure 2A. The performance of these mice across four trials suggested that the BOX maze is a valid paradigm for assessing learning and learning impairment in male mice. Female B6 mice 8 months of age showed a pattern of learning

across four separate trials in the NSD group (Figure 2B). Two-way ANOVA test suggested a significant difference in learning between the SD and NSD groups. The results also suggested there was a significant difference between two or more trials between those groups. This is visualized in the graph for females in figure 2B. The conclusion from the statistical analysis is that there was a difference in the pattern of learning between the SD and NSD groups, suggesting the BOX maze is a valid paradigm for assessing learning and learning impairment in female mice as well as male mice at a specific age.

Sleep deprived mice showed learning impairment in an age, gender and strain dependent manner.

With increasing age, female B6 mice showed a difference in learning impairment in response to sleep deprivation. Sleep deprived B6 female mice at 8 and 16-months of age showed a pattern of impairment similar to each other (Figures 2B and 3A). B6 females 22 months of age showed a delayed learning pattern showing a surprising result suggesting the mice get better with age (Figure 3B). This was significant by two-way ANOVA. The BOX maze thus showed that 8, 16 and 22-month-old female B6 mice had a significant difference between SD and NSD groups. There was also a significant difference between these two groups for two or more trials as can be seen by the p values in the graphs. The final conclusion gathered from the results is that while 8 and 16-month B6 females showed significant learning impairment as can be seen in the graph, 22-month B6 females showed a delayed learning ability. When comparing the slope of the learning curve for SD and NSD mice, it can be seen that sleep deprived mice do not learn in a manner as non-sleep deprived mice do.

When comparing BOX maze data between genders there was a significant difference between males and females. Two-way ANOVA results support the conclusion that there is a difference between NSD and SD groups between the 16-month B6 males and females (Figures 3A and 3C). There is also evidence to support a significant difference between two or more trials. The conclusion is that there was a difference in the rate of impairment of learning between the two genders. The results suggested the same between 16-month F1 males and females (Figures 4A and 4C).

BOX maze data showed a difference in sleep induced learning impairment between the two mouse strains B6 and CB6F1. CB6F1 females 16 months of age followed the same pattern of impairment as 8 and 16-month B6 females (Figures 4A, 2A and 3A, respectfully). Females, 16 months of age, showed nearly identical behavior and impairment between the two strains ($n=10$, $p<0.05$). This was shown to be significant by the two-way ANOVA test. CB6F1 female mice, 22-months of age, demonstrated a similar pattern of learning impairment compared to 16-month females (Figure 4B). Middle aged males in both strains of mice demonstrated similar learning patterns to each other (Figures 4C and 3C). When sleep deprived, the mice showed a learning behavior which fluctuated over the course of four trials. Innova showed a difference in the impairment due to sleep deprivation when comparing male and female mice. There was no significance in non-sleep deprived mice.

The Box maze showed that sleep deprived learning impairment was altered in mice treated with rapamycin

Sleep deprived mice, 22 months of age, treated with rapamycin showed improvement in learning ability compared to controls (Figure 5A). When comparing control treatment versus rapamycin treatment, the rapamycin treated mice performed significantly better than control treated mice across all four trials. When comparing treated and control treated groups, rapamycin treatment was significant. There was no significant difference between rapamycin treated sleep deprived and non-sleep deprived mice except for between trials. Control treated sleep deprived mice were found to have significant difference from rapamycin treated mice who were both sleep deprived and non-sleep deprived. There was no significant difference between control treated non-sleep deprived mice and rapamycin treated mice. There was not a significant difference in the shape of the learning pattern over the course of the four trials.

The mice from the rapamycin treatment trial were tested with the Y Maze to help assess the validity of the BOX maze. The Y maze is a commonly used tool to measure spontaneous reactions. The expectation is that an undisturbed mouse would be more likely to visit novel environments when compared to stressed mice. Two-way ANOVA showed a significant correlation between the data of the BOX maze and the Y maze (Figure 7B). This suggests that the BOX maze, when comparing escape holes visited, had a significant correlation to the arms visited in the Y maze.

Discussion

The BOX maze is an informative learning paradigm in mice, which is time effective and has multiple readouts. Its advantages lie within the amount of time it takes to conduct the experiment and the variety of results that it shows. Because the BOX maze is able to generate data on novel decision making as shown in figure 5 comparing the results of the Y maze, as well as cognition pertaining to learning, it gives an additional readout for the data. In addition, the BOX maze appears sensitive enough to distinguish between different treatments and stressors, specifically with rapamycin treatment.

The data indicate that the BOX maze is sensitive enough to distinguish between gender and strains of mice in an age-dependent manner. Mice are able to undergo this assay without endangering their health because there is little risk and less stress compared to the Morris water maze. With the increasing number of studies related to aging, the BOX maze can be a cognitive assay which is reliable and easy to use. The Box maze can distinguish between control and treated mice with the numbers of mice of different ages, strain, and gender. These results support the ability of the BOX maze to detect learning impairment in mice.

A disadvantage of the BOX maze is that mice retain the ability to remember months after the initial assay, so it is unreliable as a repetitive testing procedure. Because the BOX maze does not have a memory component, it is not effective in testing memory compared to the radial water tread maze. Fortunately, this allows for the BOX maze to be combined in a battery of cognitive behavioral tests. A mouse could have a baseline taken with the Y maze and could undergo

treatment and use the BOX maze as well as running wheel or other tests as endpoint indicators. While the data collected shows that the BOX maze is sensitive to different strains, gender and ages, testing additional age groups would have added more evidence of the ability for age-dependent assessment. While mechanisms of sleep induced learning impairment were not studied as part of this project, information pertaining to synaptic function, drug interaction, and inflammation would be of interest for a better understanding of the ability of the BOX maze to distinguish between treated and non-treated mice.

Additional studies will be able to draw conclusions on the effect of rapamycin on rescuing sleep deprivation-induced learning impairment. The BOX maze was able to visualize a significant improvement in the learning time of 22-month B6 sleep deprived mice, and there's potential for support of this conclusion through molecular analysis. The encouraging observations with rapamycin suggest the BOX maze could be an informative initial screening assay to test drugs and drug combinations for intervention of short-term sleep-deprived learning impairment.

Figure legends

Figure 1. Mockup of the BOX maze. The two gray rectangles to note the box used for the box maze as well as the escape cage. The black rectangles represent the PVC caps which are the false escape holes. The blue curve between the two mazes is the escaped tubing. During the maze, the escape cage is covered so that mice have a comfortable dark place to rest in between trials. The

singular light in the room hangs 30 inches from the bottom of the plastic box.

Figure 2. Performance of 8-month-old B6 mice, males (A) and females (B). The ANOVA analysis suggests that the sleep deprived, and non-sleep deprived groups are significantly different, there is a significant difference in two of the trials, and that the patterns of learning are significantly different between sleep deprived and non-sleep deprived mice.

Figure 3. BOX maze data in 16-month B6 females suggest that there is a significant difference between sleep deprived and non-sleep deprived mice (A). B6 females, 22 months of age, show delayed learning rather than a more severe learning impairment (B). This is backed up by a two-way ANOVA test showing a significant difference between the sleep deprived and non-sleep deprived groups, a significant difference between two or more trials, and a significant difference in the learning pattern between sleep deprived and non-sleep deprived mice. B6 males, 16 months of age, show a similar pattern as females (C).

Figure 4. ANOVA analysis showed CB6F1 mice have similar responses to sleep induced learning impairment in 16-month females (A) and males (C) and 22-month females (B) with a significant difference between sleep deprived and non-sleep deprived mice.

Figure 5. A. Two-way ANOVA analysis showed there is a difference between SD and NSD control mice (Black vs Dark Grey). It also showed a significant difference between each trial. However, it also suggested that the difference in the pattern and shape between the two groups is not enough to be significant. When comparing the rapamycin treated groups (Medium Grey vs

Light Grey) ANOVA testing showed that there is no significant difference between sleep deprived and non-sleep deprived, there is significant difference between each trial, and that the pattern and shape is similar between the two groups. When comparing data between the different Rapamycin and Control treated groups, ANOVA analysis showed a significant difference between both drug treated groups and sleep deprived vehicle group. There was a significant difference between 2 or more trials in each of these groups. ANOVA revealed that the slope of the trendline for each of the groups is very similar. This suggests that while the rapamycin treatment groups are significantly faster at learning, they are still learning at a similar rate to the vehicle injected groups. **B.** B6 female groups of mice, 22 months of age: Non-sleep deprived control (NSDC), sleep deprived control (SDC), non-sleep deprived rapamycin (NSDR), and sleep deprived rapamycin (SDR). During the test, each hole visited in the box maze was recorded as well as each arm for the Y maze. One-way ANOVA showed a significant correlation between the two mazes. This can be seen when comparing bars of the same color. The patterns seen when comparing the Y-maze to the BOX maze are identical and match the pattern seen in the Box maze data (Figure 5A).

Acknowledgments

This work was funded by NIA grant R01 AG057381 (Ladiges PI)

References

1. June J. Pilcher, Allen I. Huffcutt, Effects of Sleep Deprivation on Performance: A Meta-Analysis, *Sleep*, Volume 19, Issue 4, June 1996, Pages 318–326, https://doi.org/10.1093/sleep/19.4.318
2. Ericsson, Aaron C et al. "A brief history of animal modeling." *Missouri medicine*vol. 110,3 (2013): 201-5.
3. Wolf, Andrea et al. "A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice." *PloS one* vol. 11,1 e0147733. 25 Jan. 2016, doi:10.1371/journal.pone.0147733
4. MATER METHODS 2012;2:122
5. Pettan-Brewer etal. (2013). A novel radial water tread maze tracks age-related cognitive decline in mice. Pathobiology of aging & age related diseases. 3. 10.3402/pba.v3i0.20679.
6. Gosselin, Dominique et al. "Novel Measures to Assess the Effects of Partial Sleep Deprivation on Sensory, Working, and Permanent Memory." *Frontiers in psychology* vol. 8 1607. 28 Sep. 2017, doi:10.3389/fpsyg.2017.01607
7. Learning and Behavior tests https://med.stanford.edu/sbfnl/services/bm/lm.html
8. Prevalence of Healthy Sleep Duration among Adults--United States, 2014. Liu Y, Wheaton AG, Chapman DP, Cunningham TJ, Lu H, Croft JB
9. Colavito V, Fabene PF, Grassi-Zucconi G, et al. Experimental sleep deprivation as a tool to test memory deficits in rodents. *Frontiers in Systems Neuroscience*. 2013;7:106. doi:10.3389/fnsys.2013.00106.
10. Laurent Brondel, Michael A Romer, Pauline M Nougues, Peio Touyarou, Damien Davenne, Acute partial sleep deprivation increases food intake in healthy men, *The American Journal of Clinical Nutrition*, Volume 91, Issue 6, June 2010, Pages 1550–1559, https://doi.org/10.3945/ajcn.2009.28523
11. Paruthi S, Brooks LJ, D'Ambrosio C, et al. Recommended amount of sleep for pediatric populations: a consensus statement of the American Academy of Sleep Medicine. *J Clin Sleep Med.* 2016;12(6):785–786.
12. Wheaton AG, Olsen EO, Miller GF, Croft JB. Sleep duration and injury-related risk behaviors among high school students — United States, 2007–2013. *MMWR Morb Mortal Wkly Rep.* 2016;65:337-341. https://www.cdc.gov/mmwr/volumes/65/wr/mm6513a1.htm
13. Institute of Medicine (US) Committee on Sleep Medicine and Research; Colten HR, Altevogt BM, editors. Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Washington (DC): National Academies Press (US); 2006. 2, Sleep Physiology. Available from: https://www.ncbi.nlm.nih.gov/books/NBK19956/
14. Besedovsky, Luciana et al. "Sleep and immune function." *Pflugers Archiv : European journal of physiology* vol. 463,1 (2012): 121-37. doi:10.1007/s00424-011-1044-0
15. Graves, Laurel A et al. "Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning." *Learning & memory (Cold Spring Harbor, N.Y.)* vol. 10,3 (2003): 168-76. doi:10.1101/lm.48803
16. J Alzheimers Dis. 2016;50(3):669-85. doi: 10.3233/JAD-150774.
17. Medic, Goran et al. "Short- and long-term health consequences of sleep disruption." *Nature and science of sleep* vol. 9 151-161. 19 May. 2017, doi:10.2147/NSS.S134864

18. de Vivo L, Nelson AB, Bellesi M, Noguti J, Tononi G, Cirelli C. Loss of sleep affects the ultrastructure of pyramidal neurons in the adolescent mouse frontal cortex. Sleep (2016) 39:861–74.10.5665/sleep.5644 [PMC free article] [PubMed] [CrossRef] [Ref list]
19. Knutson, Kristen L et al. "The metabolic consequences of sleep deprivation." *Sleep medicine reviews* vol. 11,3 (2007): 163-78. doi:10.1016/j.smrv.2007.01.002
20. Am J Physiol Endocrinol Metab. 2018 Nov 1;315(5):E848-E858. doi: 10.1152/ajpendo.00072.2018. Epub 2018 Jul 10.
21. Silva, Regina Helena et al. "Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice." *Neuropharmacology* 46 (2004): 895-903.
22. Life Sci. 1996;58(5):373-95.
23. Ehninger, Dan et al. "Longevity, aging and rapamycin." *Cellular and molecular life sciences : CMLS* vol. 71,22 (2014): 4325-46. doi:10.1007/s00018-014-1677-1
24. Yiqiang Zhang, Alex Bokov, John Gelfond, Vanessa Soto, Yuji Ikeno, Gene Hubbard, Vivian Diaz, Lauren Sloane, Keith Maslin, Stephen Treaster, Samantha Réndon, Holly van Remmen, Walter Ward, Martin Javors, Arlan Richardson, Steven N. Austad, Kathleen Fischer, Rapamycin Extends Life and Health in C57BL/6 Mice, *The Journals of Gerontology: Series A*, Volume 69A, Issue 2, February 2014, Pages 119–130, https://doi.org/10.1093/gerona/glt056
25. Timo Nevalainen, Animal Husbandry and Experimental Design, *ILAR Journal*, Volume 55, Issue 3, 2014, Pages 392–398, https://doi.org/10.1093/ilar/ilu035
26. Harrison, David E et al. "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice." *Nature* vol. 460,7253 (2009): 392-5. doi:10.1038/nature08221
27. Colavito, Valeria et al. "Experimental sleep deprivation as a tool to test memory deficits in rodents." *Frontiers in systems neuroscience* vol. 7 106. 13 Dec. 2013, doi:10.3389/fnsys.2013.00106
28. Lemons, A., Saré, R. M., Beebe Smith, C. Chronic Sleep Deprivation in Mouse Pups by Means of Gentle Handling. *J. Vis. Exp.* (140), e58150, doi:10.3791/58150 (2018).
29. Taheri S, Lin L, Austin D, Young T, Mignot E; Lin; Austin; Young; Mignot (December 2004). "Short Sleep Duration Is Associated with Reduced Leptin, Elevated Ghrelin, and Increased Body Mass Index". *PLoS Med*. **1** (3): e62. doi:10.1371/journal.pmed.0010062. PMC 535701. PMID 15602591.
30. Alhola, Paula; Päivi Polo-Kantola (October 2007). "Sleep deprivation: Impact on cognitive performance". *Neuropsychiatr. Dis. Treat*. **3** (5): 553–567. PMC 2656292. PMID 19300585. Although both conditions [total and partial SD] induce several negative effects including impairments in cognitive performance, the underlying mechanisms seem to be somewhat different.
31. Patti, Camilla L et al. "Effects of sleep deprivation on memory in mice: role of state-dependent learning." *Sleep* vol. 33,12 (2010): 1669-79. doi:10.1093/sleep/33.12.1669
32. Van Hulzen ZJ, Coenen AM; Coenen (December 1980). "The pendulum technique for paradoxical sleep deprivation in rats". *Physiol. Behav*. 25 (6): 807–11. doi:10.1016/0031-9384(80)90298-X. PMID 7220622.
33. Linde, L.; Edland, A.; Bergstrom, M. (1999). "Auditory attention and multiattribute decision-making during a 33-h sleep deprivation period: mean performance and between-subject dispersions". *Ergonomics*. 42 (5): 696–713. doi:10.1080/001401399185397.
34. Cote, K. A.; Milner, C. E.; Osip, S. L.; Baker, M. L.; Cuthbert, B. P. (2008). "Physiological arousal and attention during a week of continuous sleep restriction". *Physiology & Behavior*. 95: 353–364. doi:10.1016/j.physbeh.2008.06.016.
35. Tsuno, N., Besset, A., & Ritchie, K. (2005). Sleep and Depression. *The Journal of Clinical Psychiatry, 66*(10), 1254-1269. http://dx.doi.org/10.4088/JCP.v66n1008

36. Watson NF, Badr MS, Belenky G, et al.; Consensus Conference Panel. Joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society on the recommended amount of sleep for a healthy adult: methodology and discussion. *Sleep*. 2015;38:1161–1183.
37. Watson NF, Badr MS, Belenky G, et al.; Consensus Conference Panel. Joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society on the recommended amount of sleep for a healthy adult: methodology and discussion. *Sleep*. 2015;38:1161–1183.
38. Paruthi S, Brooks LJ, D'Ambrosio C, et al. Recommended amount of sleep for pediatric populations: a consensus statement of the American Academy of Sleep Medicine. *J Clin Sleep Med*. 2016;12(6):785–786.
39. Erasing Synapses in Sleep: Is It Time to Be SHY? Marcos Gabriel Frank. Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6074, USA. Received 18 October 2011; Accepted 4 December 2011
40. R. Basheer, R. Brown, V. Ramesh, S. Begum, and R. W. McCarley, "Sleep deprivation-induced protein changes in basal forebrain: implications for synaptic plasticity," Journal of Neuroscience Research, vol. 82, no. 5, pp. 650–658, 2005. View at Publisher · View at Google Scholar · View at Scopus
41. M. Neuner-Jehle, T. A. Rhyner, and A. A. Borbely, "Sleep deprivation differentially alters the mRNA and protein levels of neurogranin in rat brain," Brain Research, vol. 685, no. 1-2, pp. 143–153, 1995. View at Publisher · View at Google Scholar · View at Scopus
42. Z. Guan, X. Peng, and J. Fang, "Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus," Brain Research, vol. 1018, no. 1, pp. 38–47, 2004. View at Publisher · View at Google Scholar · View at Scopus
43. P. Taishi, C. Sanchez, Y. Wang, J. Fang, J. W. Harding, and J. M. Krueger, "Conditions that affect sleep alter the expression of molecules associated with synaptic plasticity," American Journal of Physiology, vol. 281, no. 3, pp. R839–R845, 2001. View at Google Scholar · View at Scopus
44. Methods Mol Biol. 2019;1916:105-111. doi: 10.1007/978-1-4939-8994-2_10.
45. Wolf, Andrea et al. "A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice." *PloS one* vol. 11,1 e0147733. 25 Jan. 2016, doi:10.1371/journal.pone.0147733
46. Bitto, Alessandro et al. "Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice." *eLife* vol. 5 e16351. 23 Aug. 2016, doi:10.7554/eLife.16351
47. Kim, Hae-Young. "Analysis of variance (ANOVA) comparing means of more than two groups." *Restorative dentistry & endodontics* vol. 39,1 (2014): 74-7. doi:10.5395/rde.2014.39.1.74

Figures

Figure 1

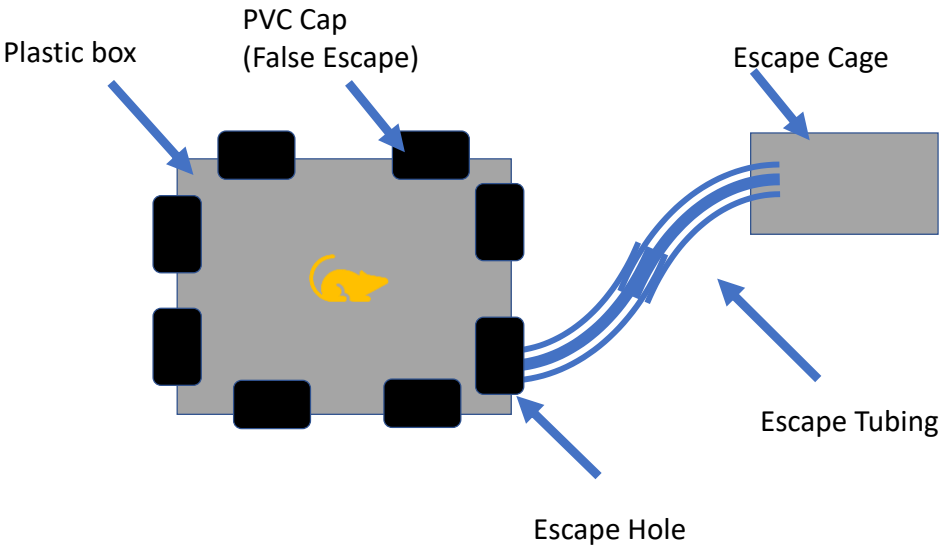


Figure 2

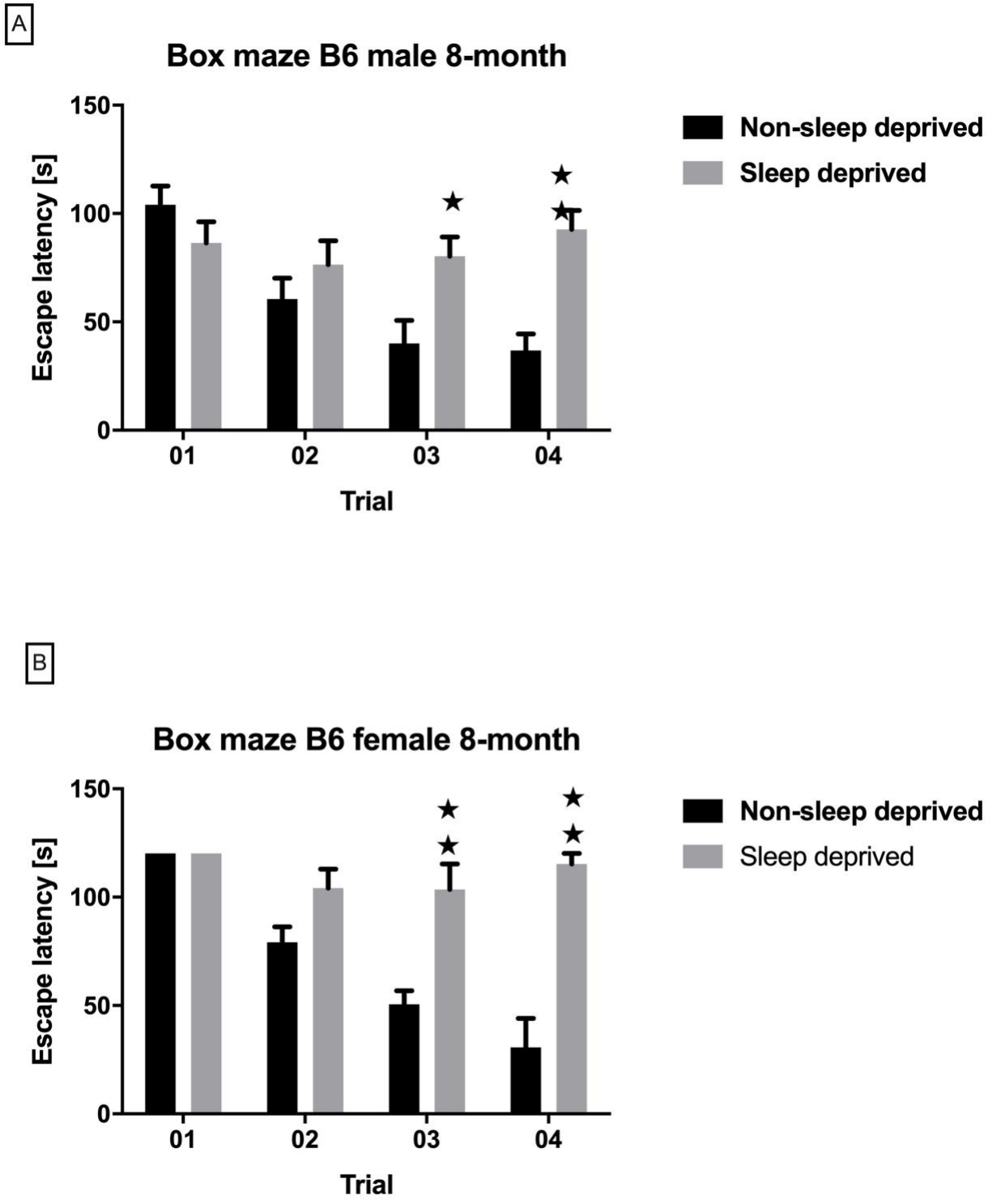

A
Box maze B6 male 8-month
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
Non-sleep deprived
Sleep deprived
B
Box maze B6 female 8-month
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
Non-sleep deprived
Sleep deprived

Figure 3

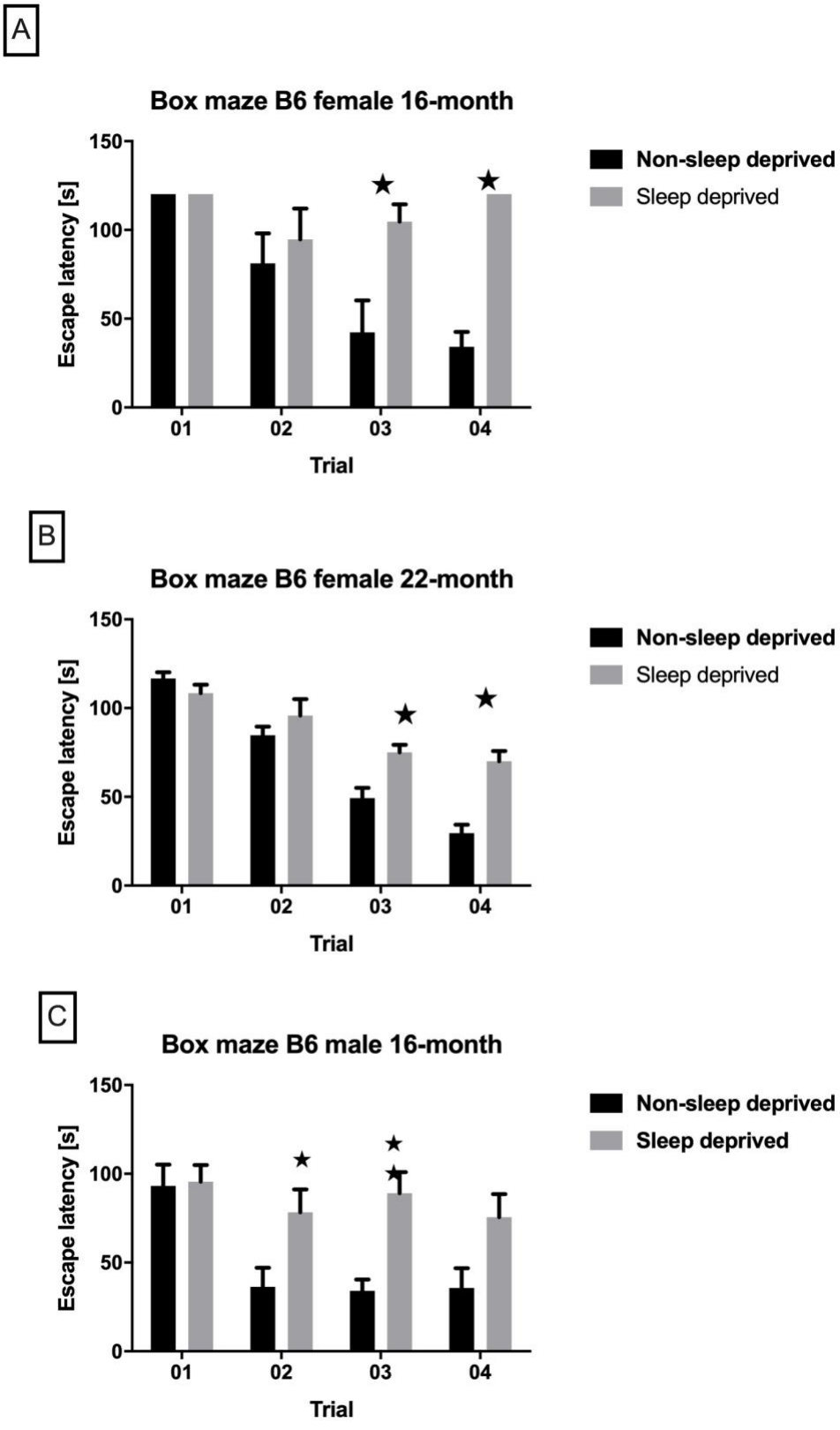

A
Box maze B6 female 16-month
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
Non-sleep deprived
Sleep deprived
B
Box maze B6 female 22-month
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
Non-sleep deprived
Sleep deprived
C
Box maze B6 male 16-month
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
Non-sleep deprived
Sleep deprived

Figure 4

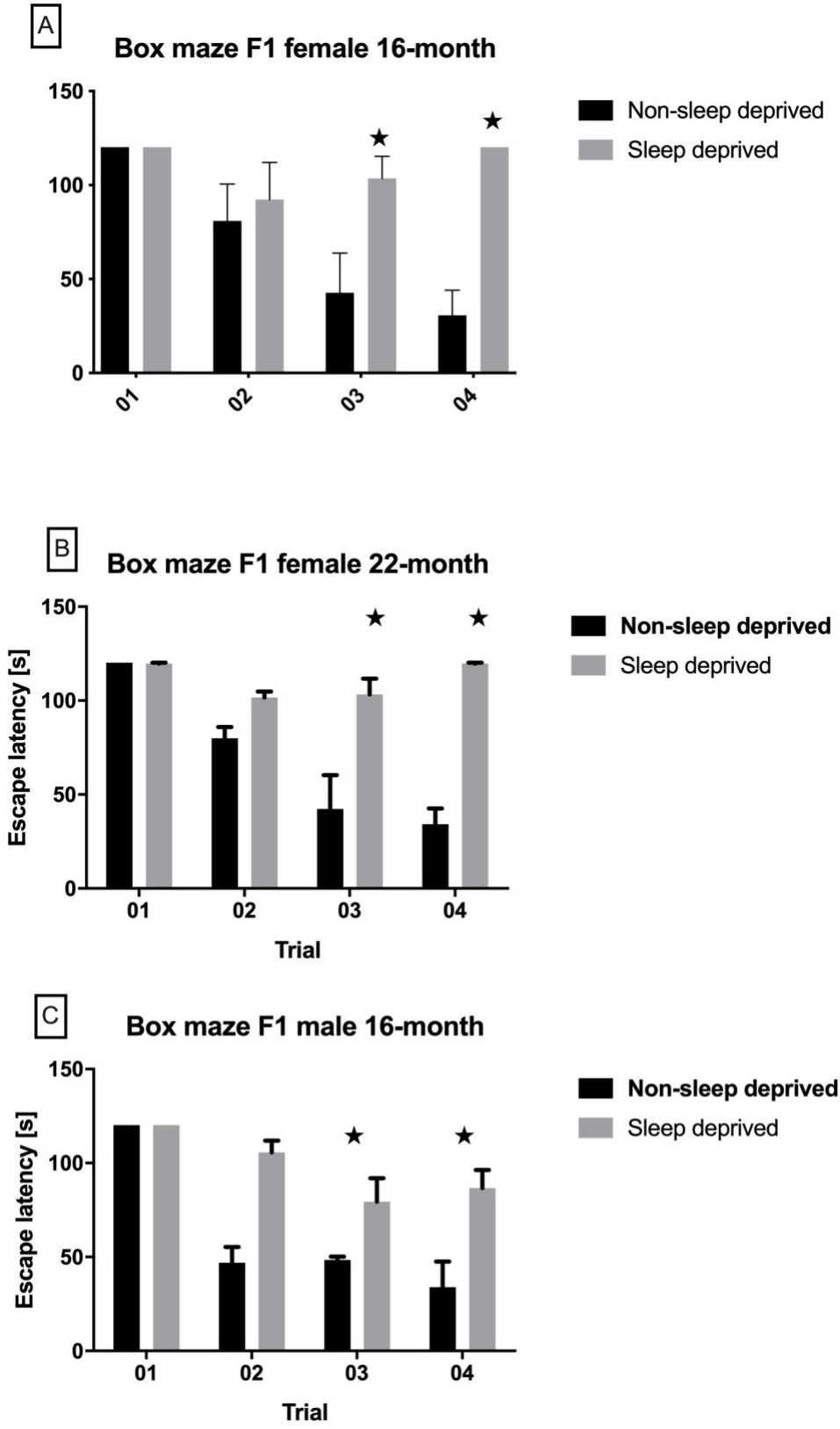
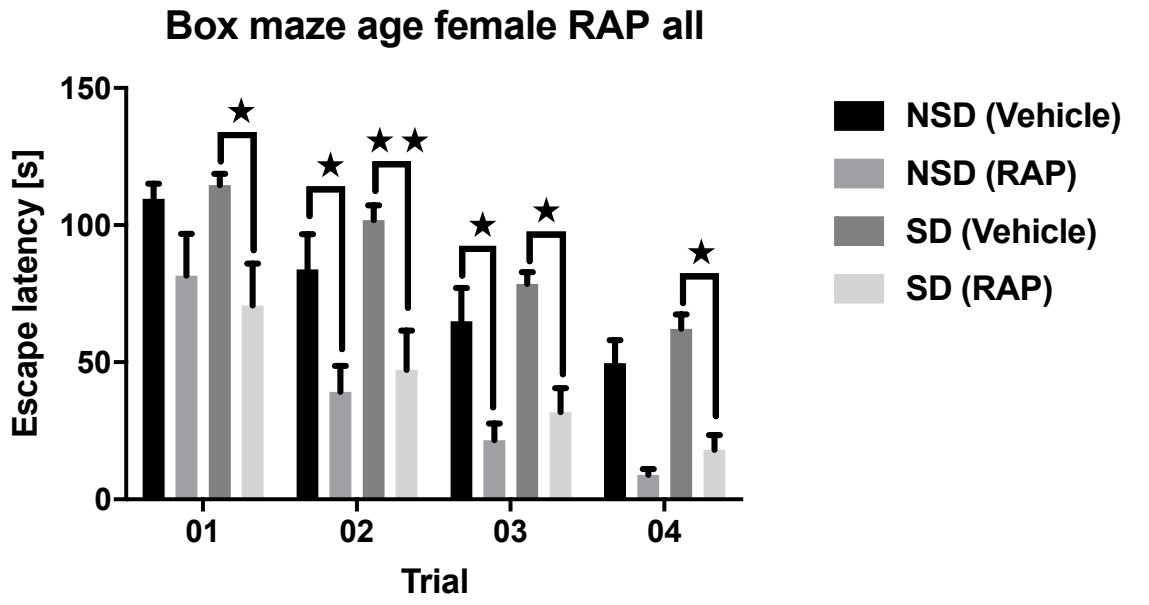
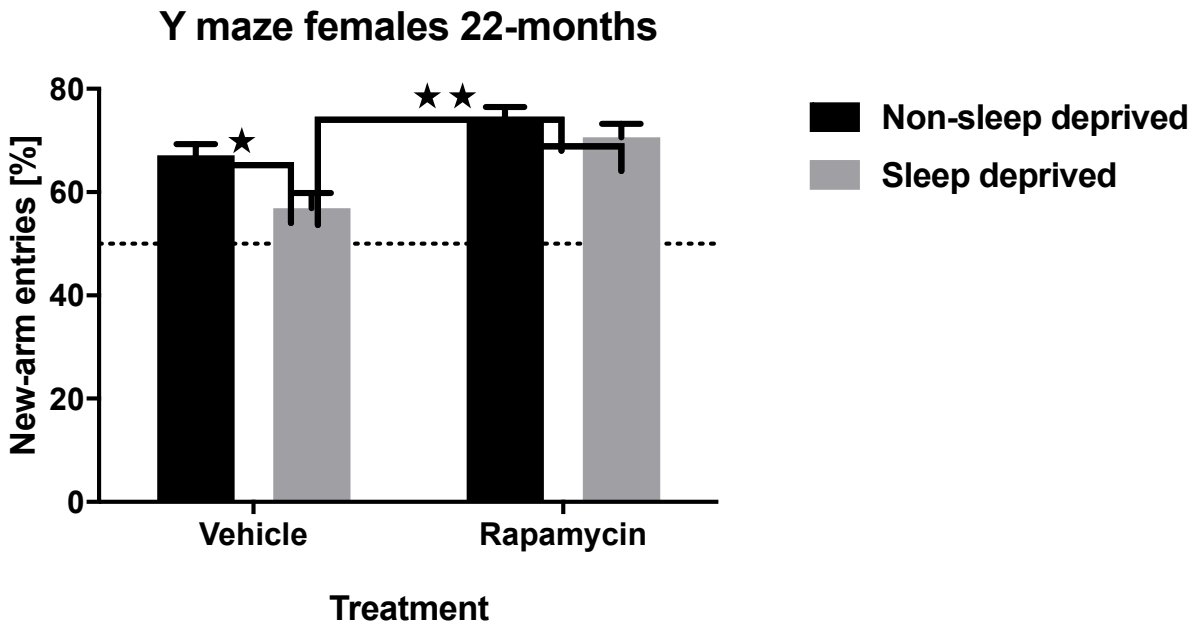

A
Box maze F1 female 16-month
150
100
50
0
01
02
03
04
Non-sleep deprived
Sleep deprived
B
Box maze F1 female 22-month
Escape latency [s]
Trial
C
Box maze F1 male 16-month

Figure 5

A


Box maze age female RAP all
Escape latency [s]
150
100
50
0
01
02
03
04
Trial
NSD (Vehicle)
NSD (RAP)
SD (Vehicle)
SD (RAP)

B


Y maze females 22-months
New-arm entries [%]
80
60
40
20
0
Vehicle
Rapamycin
Treatment
Non-sleep deprived
Sleep deprived