



INTRODUCTION

Background

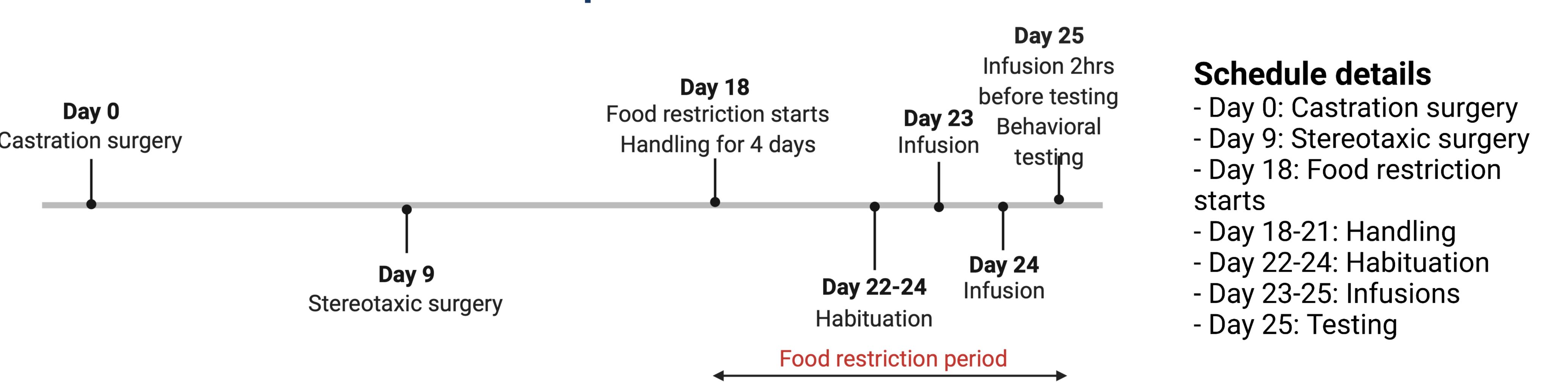
- Spatial memory has been found to decline significantly in older populations of both humans and rodent (Erickson & Barnes, 2003, Shukitt-Hale et al., 2004)
- Testosterone is known to play a significant role in learning as androgen receptors are found abundant in areas of learning and memory in the brain such as the hippocampus (Menard and Harlan, 1993)
- However, studies on the benefits of testosterone replacement on spatial memory have provided mixed results
- This inconsistency may be explained by the dose-dependent effects of testosterone on the brain. Previous work in our lab demonstrated that a high dose of subcutaneous testosterone improves place learning while a low dose improves response tasks (Zhang et al., 2020)
- Previous studies have found that the dorsal hippocampus is important for place learning while the dorsal striatum is important for response learning (Iaria et al., 2003, Moser et al., 1993)
- This project aims to answer the question of whether testosterone affects spatial memory by acting directly upon cells in specific regions of the brain rather than through a more indirect route.

Hypothesis

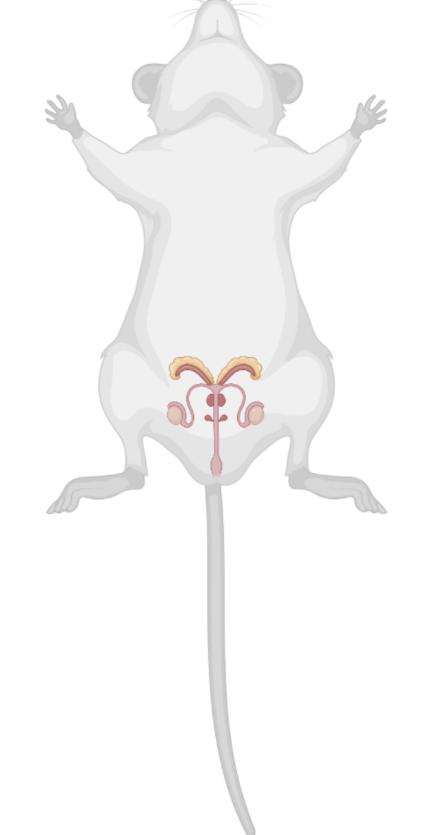
Low dose of testosterone activates the response learning strategy by direct action in the striatum and a high dose of testosterone activates the cells in the hippocampus.

METHODS

General procedure

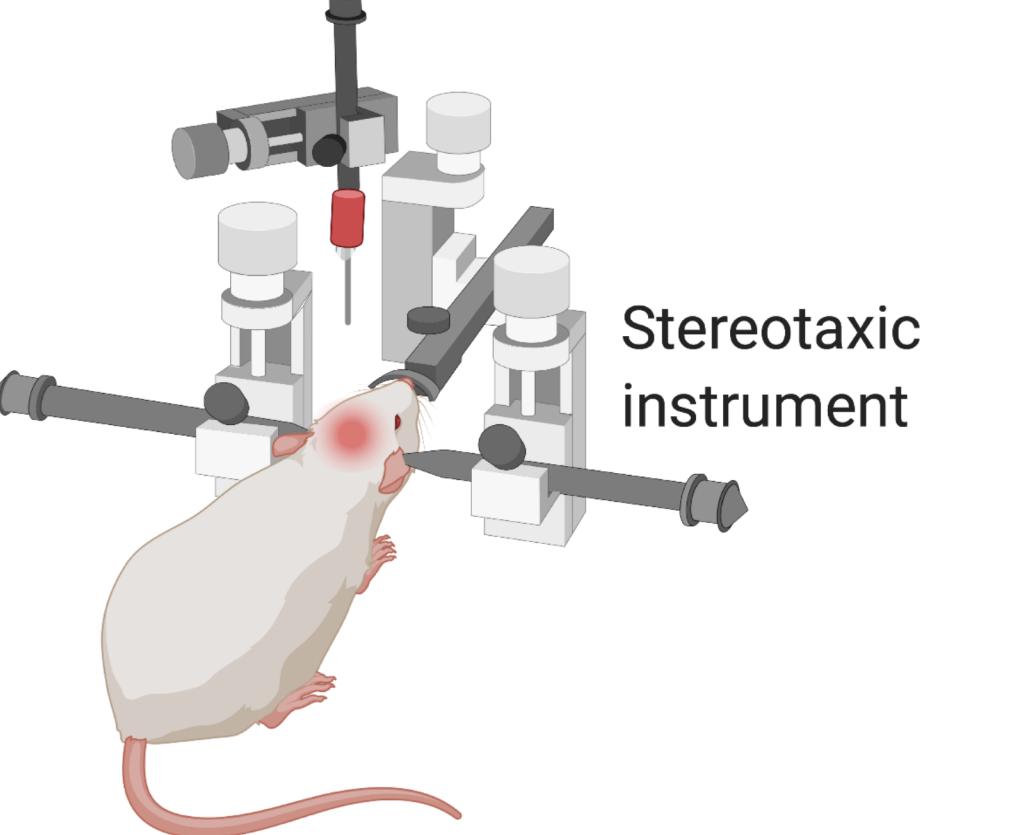


Castration surgery



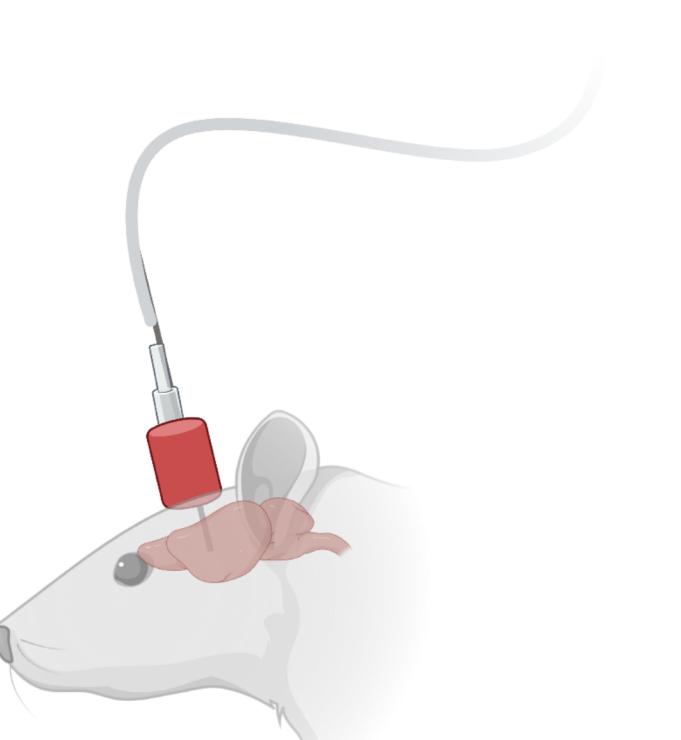
- Approximately 60 days of Fischer rats were used
- All rats were bilaterally castrated under isoflurane anesthesia (2.0-3.5%) and allowed to recover for 7-8 days

Stereotaxic surgery



- After castration surgeries, subjects underwent STX surgery under isoflurane (2.0-3.5%) to implant bilateral cannula guides into either:
- Dorsal hippocampus (AP = -3.88 mm, ML = 2.5 mm, DV: 1.9 mm)
- Dorsal striatum (AP: 0.2 mm, ML: 3.6 mm, DV: 2.8 mm)

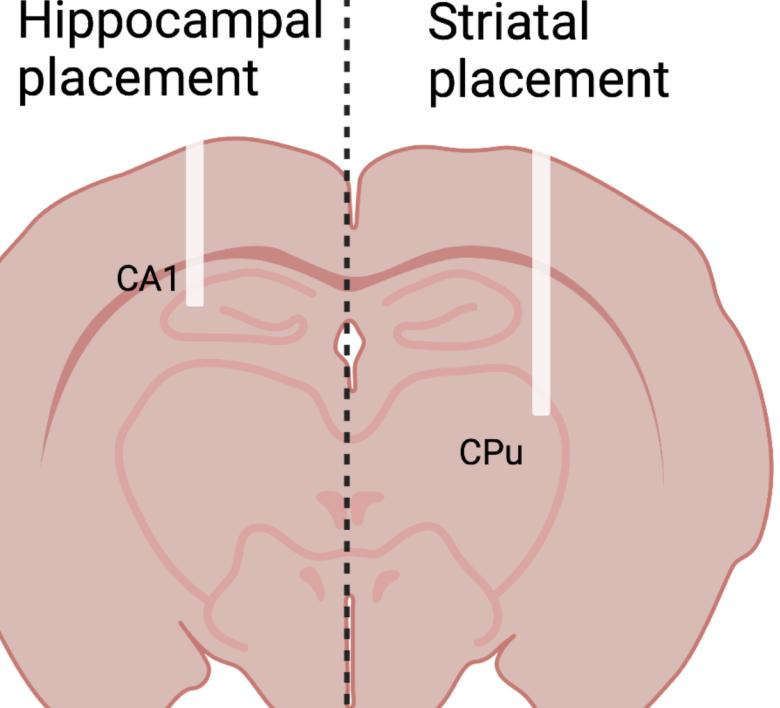
Intracerebral infusion



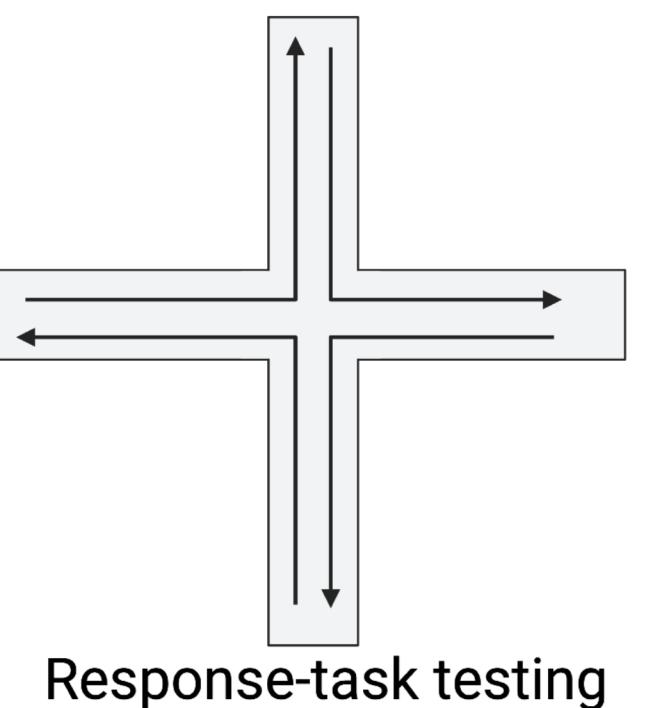
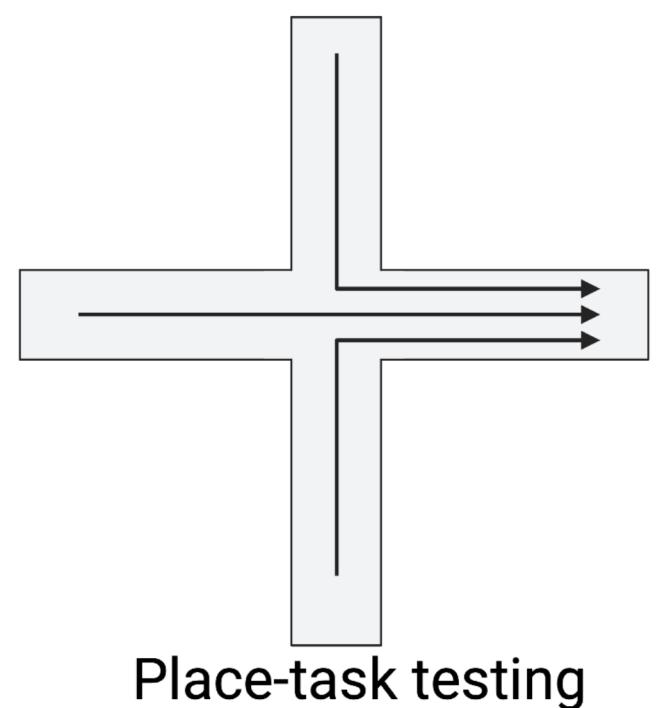
- Subjects received three intrahippocampal/intrastriatal injections of DMSO or testosterone dissolved in DMSO at 48 hours, 24 hours and 2 hours before training.
- For place learning:
DMSO 0.5 μ l/hemisphere (n=14) 0.1 μ g T/hemisphere (n=13)
1.0 μ g T/hemisphere (n=13) 10.0 μ g T/hemisphere (n=12)

Cannula placement verification

- Each rat was euthanized by an i.p. injection of a lethal dose of sodium pentobarbital
- The subjects were transcardially perfused with 60ml of 0.9% saline then 120 ml of 4% (v/v) paraformaldehyde PFA
- Brains were sliced into 40 μ m coronal sections using a vibrating microtome (Leica VT1000S) on a chilled TBS bath from rostral to caudal
- Brain tissues were stained with Cresyl Violet (Fischer) and examined for cannula placement under a light microscope



- Extra cues as high contrast posters were mounted on the walls of the testing room
- The behavioral testing protocol includes four days of handling, three days of habituation (which began three days before testing), and one day of either place learning or response learning testing.
- Habituation day 1: explore maze for 5 minutes. Day 2: Explore maze with 3 pellets each maze.
- Day 3: Explore maze with pellet on the reward cup at the end of maze arm.
- Testing day: For the place learning strategy, rats were trained to locate the reward pellet that would be placed in a consistent arm relative to the extra-maze cues. For rats assigned to motor-response learning trials, rats were trained to locate the reward pellet that was consistently in the left or right relative to their body



RESULTS

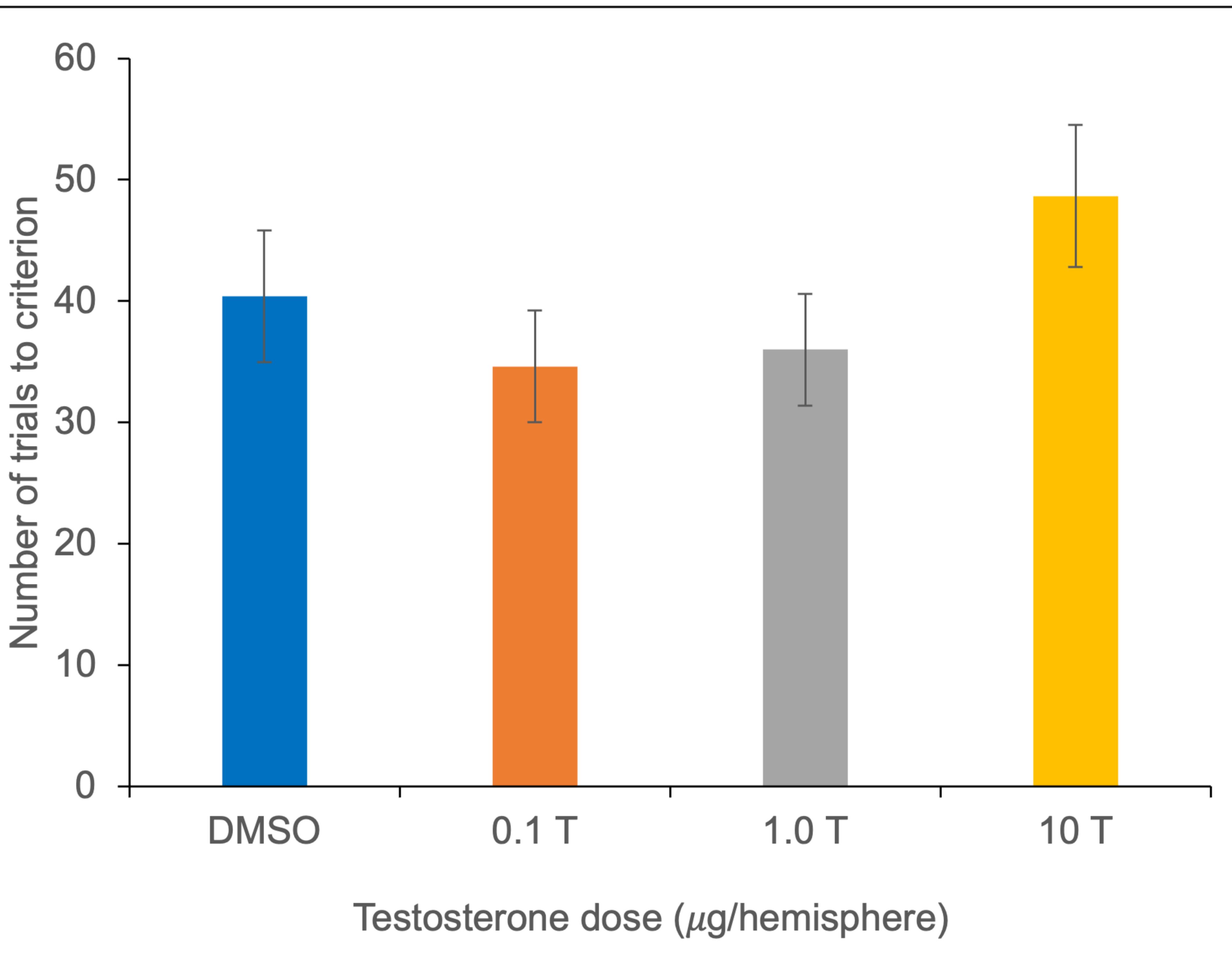


Figure 1. Means of trials to criterion (+/- SEM) during place testing for rats receiving either DMSO or testosterone injections (n=12-14/group) 48 hours, 24 hours and 2 hours before training. There was no significant effect of dosing treatment on the mean of number of trials to reach criterion (correct 9 out of 10 trials), $F(3,48) = 1.493$, $p = 0.228$.

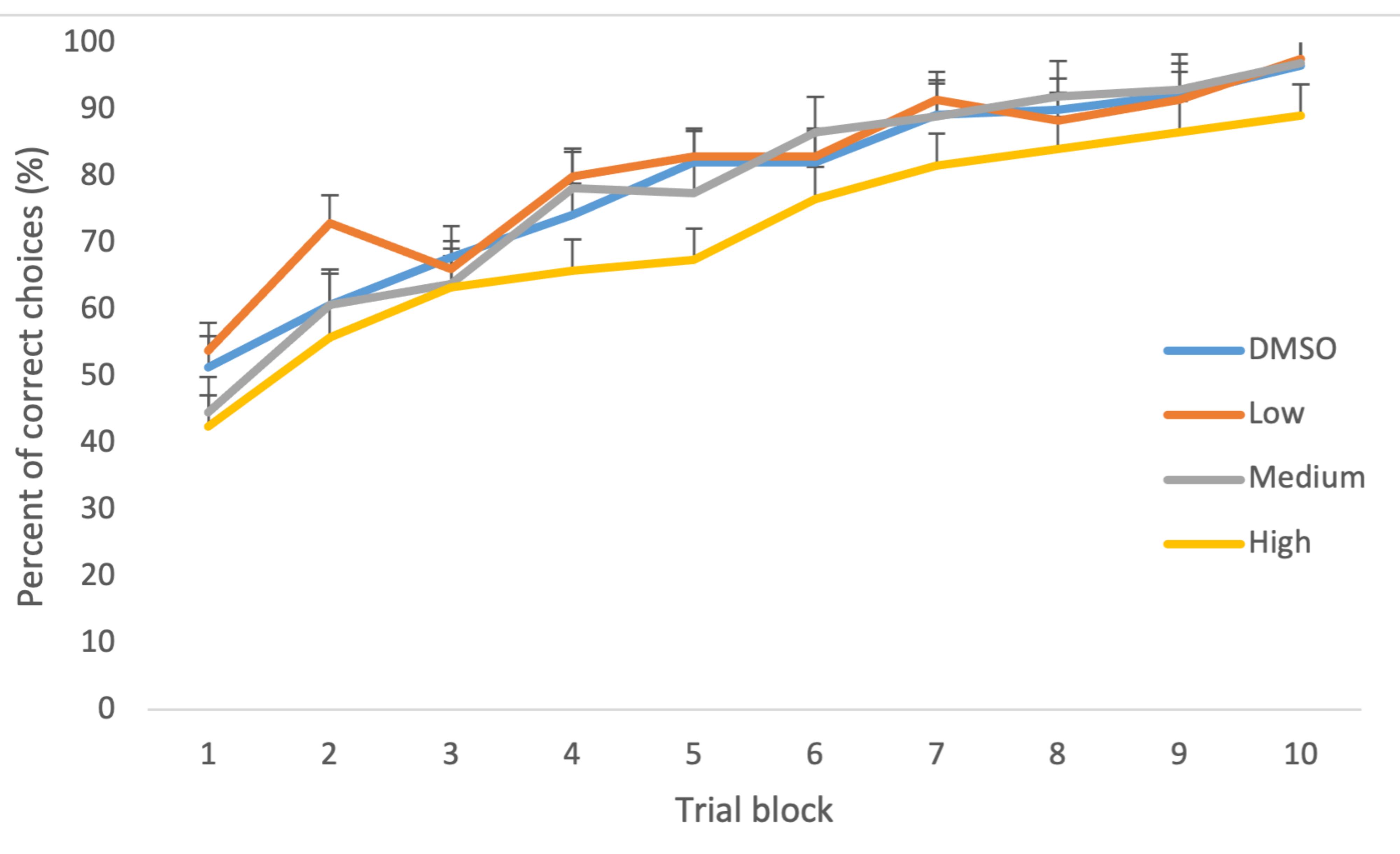


Figure 2. Mean number of correct arm choice (+/- SEM) every 10-trial block for rats receiving either DMSO or testosterone injections (n=12-14/group) 48 hours, 24 hours and 2 hours before training. Repeated measure ANOVA reveals that there was a significant main effect of different testosterone treatments and percent of correct choices over time, $F(3,45) = 4076.973$, $p < 0.001$. Post-hoc LSD suggests that rats receiving the high dose of testosterone significantly impaired place memory compared to the DMSO control group, $p = 0.036$.

CONCLUSION

Effects of testosterone on place learning

- There was no significant effect of testosterone dosing on the mean number of trials to complete criterion. However, we detected a main effect of testosterone dosing treatments on learning curves. This can happen as learning curves break the learning into smaller blocks and document how each group improves memory performance over time.
- High dose of testosterone (10 μ g testosterone/hemisphere) significantly impaired rats' performance on place learning compared to the control group.
- Low (0.1 μ g testosterone/hemisphere) and medium dose (1.0 μ g testosterone/hemisphere) have no significant effects on place learning. However, there is a trend that low dose of testosterone improved place learning while rats with the medium dose performed poorly on the task.
- This result contradicts previous studies from our lab which found that a high dose of s.c. injection of testosterone improves place learning.
- This result implicates that the effects of testosterone on place learning may be mediated through an indirect route.

ACKNOWLEDGEMENTS

- I would like to thank both of my wonderful mentors, Dr. Mark Spritzer and Dr. Michael Dash, for their immense wisdom and guidance.
- I would like to thank everyone in the Spritzer lab for assisting me on this project. I want to acknowledge the help of Nicole Grullon and Ugo Iroh in assisting with surgeries. Tinglin Shi, Mark and Rasika Iyer for helping me with some castration surgeries. Em Luber for feeding my rats.
- I would also like to acknowledge the help of our vivarium staff, specifically Megan Warner-Hough, Eliza Gardner-Morse and Nina Houser, who work every day to make sure that our animals are in the best shape. In addition, I would like to thank Megan for her emotional and professional support when I was in trouble.

BIBLIOGRAPHY

- Erickson, C.A., Barnes, C.A., 2003. The neurobiology of memory changes in normal aging. *Experimental Gerontology*, *Proceedings of the 6th International Symposium on the Neurobiology and Neuroendocrinology of Aging* 38, 61–69. [https://doi.org/10.1016/S0531-5565\(02\)00160-2](https://doi.org/10.1016/S0531-5565(02)00160-2)
- Iaria, G., Petrides, M., Dagher, A., Pike, B., Bohbot, V.D., 2003. Cognitive Strategies Dependent on the Hippocampus and Caudate Nucleus in Human Navigation: Variability and Change with Practice. *J. Neurosci.* 23, 5945–5952. <https://doi.org/10.1523/JNEUROSCI.23-13-05945.2003>
- Menard, C.S., Harlan, R.E., 1993. Up-regulation of androgen receptor immunoreactivity in the rat brain by androgenic-anabolic steroids. *Brain Research* 622, 226–236. [https://doi.org/10.1016/0006-8993\(93\)90823-6](https://doi.org/10.1016/0006-8993(93)90823-6)
- Moser, E., Moser, M.B., Andersen, P., 1993. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *J. Neurosci.* 13, 3916–3925. <https://doi.org/10.1523/JNEUROSCI.13-09-03916.1993>
- Shukitt-Hale, B., McEwen, J.J., Szprengiel, A., Joseph, J.A., 2004. Effect of age on the radial arm water maze—a test of spatial learning and memory. *Neurobiology of Aging* 25, 223–229. [https://doi.org/10.1016/S0197-4580\(03\)00041-1](https://doi.org/10.1016/S0197-4580(03)00041-1)
- Zhang, K.J., Ramdev, R.A., Tuta, N.J., Spritzer, M.D., 2020. Dose-dependent effects of testosterone on spatial learning strategies and brain-derived neurotrophic factor in male rats. *Psychoneuroendocrinology* 121, 104850. <https://doi.org/10.1016/j.psyneuen.2020.104850>