Instating Natural Reward-Induced Conditioned Place Preference in C57BL/6J Mice

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Primary Research Question

Do C57BL/6J mice develop conditioned place preference to a chocolate-paired conditioning context?

Background

Reward processing involves the combination of intrinsic and environmental cues to induce systematic and well-organized neurotransmitter and brain region interactions to regulate behaviors such as avoidance, prediction, goal-directed behavior, and adaptive behavior[2, 4, 5, 6, 8]. Integrating diverse brain regions, such as the ventral tegmental area (VTA), lateral habenula (LHb), nucleus accumbens (NAc), dopamine has been found to be the primary neurotransmitter modulating reward and associated processes. Behaviorally, reward can be studied through the conditioned place preference (CPP) model, which involves pairing a conditioning context with a stimulus, then analyzing preference. The present study seeks to investigate natural reward in rodents using five milk chocolate chips as the stimulus in a CPP paradigm. It was hypothesized that the experimental mice would develop CPP to the chocolate-paired conditioning context.

Rationale

Reward is a universally relevant field of research that can encompass ideas from understanding addiction to why we enjoy the foods that we do. The study of reward can be applied to many facets of neuroscience and psychology, such as addiction, drug-dependent behavior, aversion, and relapse. Through studies utilizing drugs of abuse, such as methamphetamines, cocaine, and alcohol, we have made great advancements in our understanding of conditions which manifest in dysfunctional reward circuitry, such as post-traumatic stress disorder (PTSD), attention-deficit/hyperactivity disorder (ADHD), substance use disorder (SUD) and depressive disorders.

Methodology

Conditioned Place Preference

Sixteen C57BL/6J mice were subjected to a ten-day CPP paradigm, beginning with a habituation phase on Day 1 and subsequently eight days of ten-minute conditioning trials. Five milk chocolate chips were present in the conditioning chamber on CS+ days, which then alternated with CS- days. On the CS- days, no additional stimuli were present in the conditioning chamber. On day ten, the preference test occurred, where percent preference data was collected (Fig. 1).

On CS+ days, the chocolate ships were weighed before and after the trial to obtain reward consumption data. During the preference test, the number of seconds spent on the CS+ side was converted to a percent preference for data analysis.

Figure 1

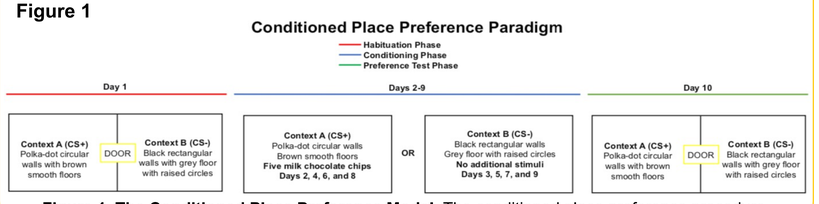

Figure 1. The Conditioned Place Preference Model. The conditioned place preference procedure utilized in the present study with the contents of each conditioning environment. It is of note that the conditioning contexts presented in the Habituation Phase and the Preference Test were the same, and that the Conditioning Phase was the only phase during which the reward was available.

Results

Figure 2

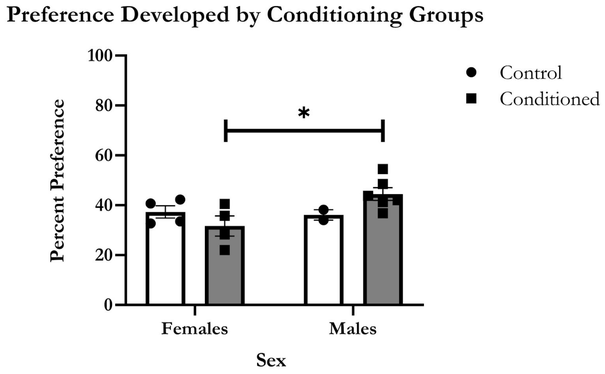

Figure 2. Preference by Sex and Treatment Group. No main effects of conditioning nor sex were found. A conditioning by sex interaction, however, was trending toward significance. When comparing experimental males to experimental females, the males displayed higher percent preference for the CS+ context, suggesting that the CPP procedure was more effective in the male subjects than the female subjects. *p = 0.035, Tukey *post hoc* analysis

Figure 3

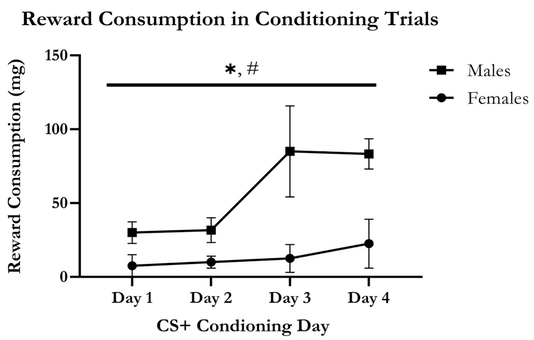

Figure 3. Chocolate Consumption by Experimental Mice Throughout Conditioning. For all trials, the average amount of chocolate consumed was higher in the male mice than in the female mice, as supported by a significant within subjects main effect of Conditioning Day. Furthermore, a significant between subjects main effect of Sex emphasizes the variation between the males and females in consumption. *, main effect of Conditioning Day, p = 0.035, Repeated Measures ANOVA; #, main effect of Sex, p = 0.021, Repeated Measures ANOVA

Summary

Percent Preference for Chocolate-Paired Conditioning Context

- No differences between the conditioned and control groups were found
- The experimental male mice developed a significantly higher preference for the chocolate-paired context in comparison to experimental female mice

Consumption of Natural Reward

- Experimental male mice consumed significantly more chocolate than the experimental female mice across all CS+ conditioning days
- Experimental male mice consumed significantly different amounts of chocolate on each day of conditioning

Discussion and Conclusions

Conclusions

- Significant sex differences were found in the experimental mice, in both the preference test and reward consumption data
- Potential presence of dose-dependent effects, as the males underwent stronger conditioning and consumed more chocolate
  - Similar observations have been made in studies utilizing an unnatural rewards (nicotine, cocaine), yet such sex differences were only significant at low reward doses[1, 3, 7, 9].
- Potential differences in dopamine activity and dopaminergic system regulation due to temporal effects of the rodent estrous cycle

Limitations

- Lack of counterbalancing
- Sample size

Future Questions

- Further characterization of the neurons active during the preference test through fluorescent microscopy techniques, specifically the braking mechanism in the glutamatergic projection depicted in Figure 4
- Deeper investigation into the interaction between and effects of sex and reward dose
- Comparative study on natural and unnatural reward processing, emphasizing sex, the identity of the reward, and dose

Figure 4

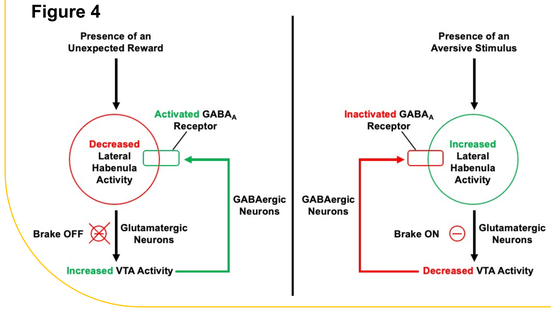

Figure 4. Braking Mechanism in the LHb-VTA Reward Circuit. This pathway displays how different stimuli regulate the activity of the LHb and, consequently, the VTA. The increase or decrease of activity in the VTA initiates a feedback loop and the braking mechanism in the glutamatergic projection from the LHb to the VTA and the GABA$_A$ receptors within the LHb. Further experiments are necessary to elucidate the activity of this pathway during reward.

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References

[1] Cullity, E. R., Guerin, A. A., Perry, C. J., & Kim, J. H. (2021). Examining sex differences in conditioned place preference or aversion to methamphetamine in adolescent and adult mice. *Frontiers in Pharmacology, 12*. https://doi.org/10.3389/fphar.2021.770614
[2] Gordon-Fennell, A., & Stuber, G. D. (2021). Illuminating subcortical GABAergic and glutamatergic circuits for reward and aversion. *Neuropharmacology*, *198*. https://doi.org/10.1016/j.neuropharm.2021.108725
[3] Hilderbrand, E. R., & Lasek, A. W. (2014). Sex differences in cocaine conditioned place preference in C57BL/6J mice. *NeuroReport*, *25*(2), 105–109. https://doi.org/10.1097/wnr.0000000000000053
[4] Mondoloni, S., Mameli, M., & Congiu, M. (2022). Reward and aversion encoding in the lateral habenula for innate and learned behaviours. *Translational Psychiatry*, *12*(1). https://doi.org/10.1038/s41398-021-01774-0
[5] Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nature Reviews Neuroscience*, *18*(2), 73–85. https://doi.org/10.1038/nrn.2016.165
[6] Rademacher, L., Schulte-Rüther, M., Hanewald, B., & Lammertz, S. (2015). Reward: From basic reinforcers to anticipation of social cues. *Social Behavior from Rodents to Humans*, 207–221. https://doi.org/10.1007/7854_2015_429
[7] Russo, S. J., Jenab, S., Fabian, S. J., Festa, E. D., Kemen, L. M., & Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. *Brain Research*, *970*(1-2), 214–220. https://doi.org/10.1016/s0006-8993(03)02346-1
[8] Saunders, B. T., Richard, J. M., & Janak, P. H. (2015). Contemporary approaches to neural circuit manipulation and mapping: Focus on reward and addiction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1677). https://doi.org/10.1098/rstb.2014.0210
[9] Yararbas, G., Keser, A., Kanit, L., & Pogun, S. (2010). Nicotine-induced conditioned place preference in rats: Sex differences and the role of mglur5 receptors. *Neuropharmacology*, *58*(2), 374–382. https://doi.org/10.1016/j.neuropharm.2009.10.001