Anxiolytic-like effect of diazepam in the novelty-suppressed feeding and novelty-induced hypophagia assays – exploring sex differences in C57BL/6J mice

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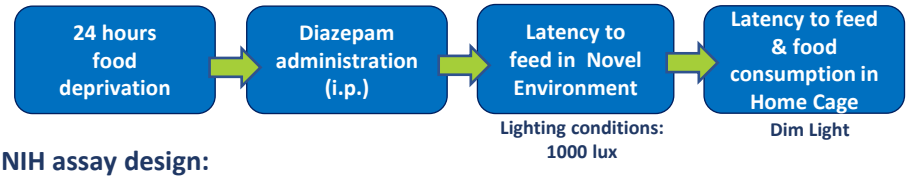
Introduction

Anxiety disorders are presently the most predominant psychiatric disorders worldwide and their incidence is still increasing. Recent studies indicate that women exhibit a higher prevalence of anxiety disorders compared to men [1]. Despite this, preclinical research has an inherent and persistent bias towards male animals, resulting in limited availability of validated animal models for both sexes including studies on anxiety and stress disorders [2]. Therefore, here we investigated sex differences in response to diazepam in two anxiety-related assays based on hyponeophagia: Novelty Suppressed Feeding (NSF) and Novelty-Induced Hypophagia (NIH) in C57BL/6J mice.

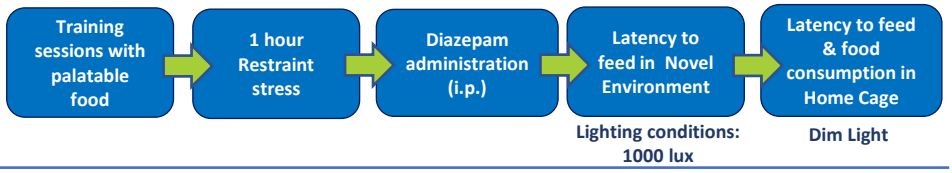
Methods

The study involved administering diazepam at three different doses (0.5, 1.0, 1.5 mg/kg, i.p.) to female and male C57BL/6J mice (8-9 weeks old) in both NSF and NIH assays. The mice were then placed in a novel environment (1000 lux light intensity) with either food pellets or palatable food, and the latency to feed was measured for a maximum of 10 minutes. After the novel environment trial, a 5-minute home cage test (50 lux light intensity) was conducted to assess latency to feed and food consumption.

NSF assay design:

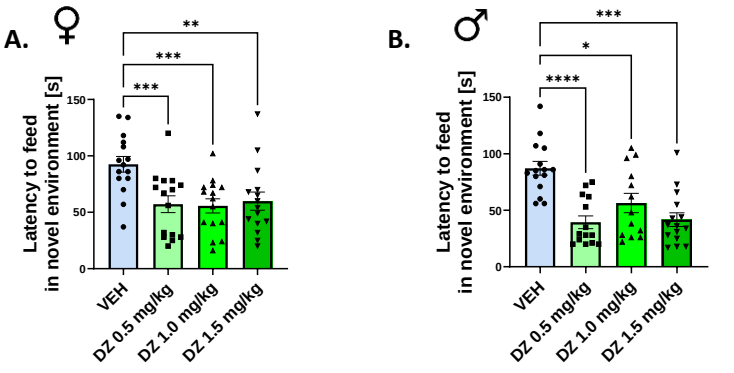


NIH assay design:



Results

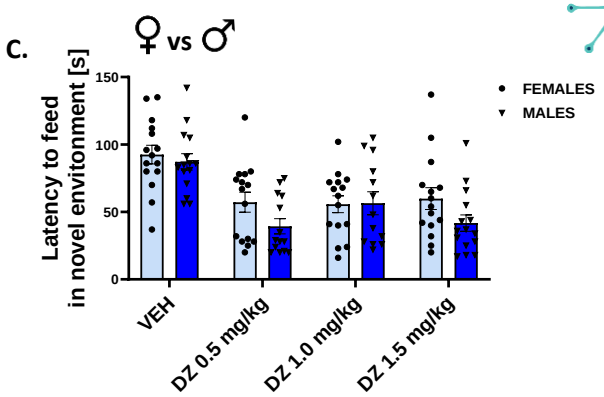
I. Effect of diazepam on Latency to feed in the novel environment in the NSF test:



Diazepam decreased latency to feed at all tested doses in (A) females and (B) males.

n= 15 per group. Mean ± SEM, **p<0.01, ***p<0.001, One way ANOVA followed by Uncorrected Fisher's LSD test.

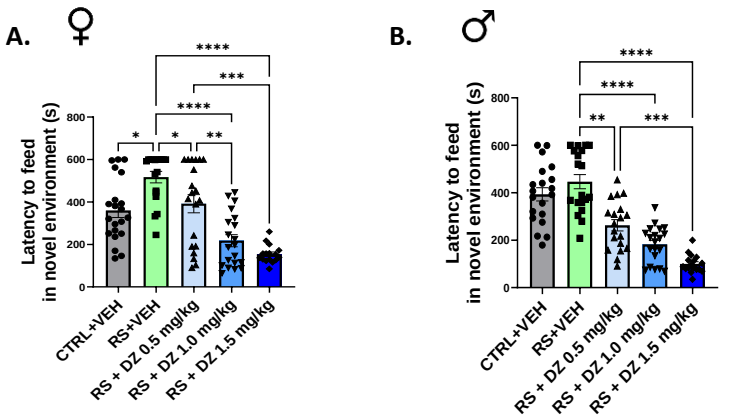
n= 13-15 per group. Mean ± SEM, ***p<0.001, Kruskal-Wallis test followed by Uncorrected Dunn's test.



C. Diazepam effect on anxiety-like behaviour in NSF test showed no sex differences.

n= 13-15 per group. Mean ± SEM. Two way ANOVA followed by Uncorrected Fisher's LSD test.

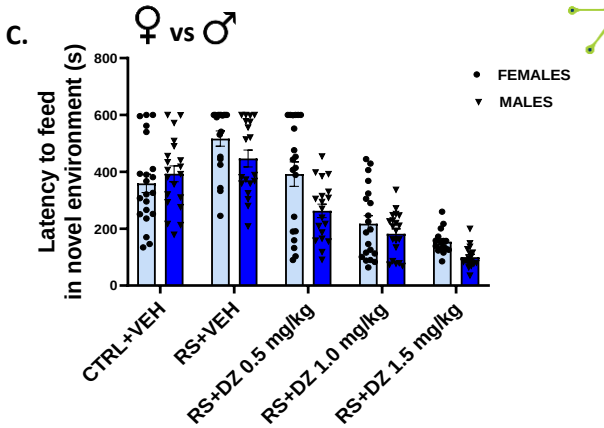
II. Effect of diazepam on Latency to feed in the novel environment in the NIH test:



Diazepam decreased latency to feed at all tested doses in (A) females and (B) males. Females exhibited increased sensitivity to restraint (A).

n= 16-21 per group. Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Kruskal-Wallis followed by Uncorrected Dunn's test.

n= 17-20 per group. Mean ± SEM, **p<0.01, ***p<0.001, ****p<0.0001 Kruskal-Wallis test followed by Uncorrected Dunn's test.



C. Diazepam effect on anxiety-like behaviour in NIH test showed no sex differences.

n= 16-20 per group. Mean ± SEM. Two way ANOVA followed by Uncorrected Fisher's LSD test.

VEH: vehicle **DZ**: diazepam **RS**: restraint stress

Conclusions

- Acute diazepam administration demonstrated robust anxiolytic efficacy in both female and male C57Bl/6J mice in the NSF and NIH assays, as evidenced by reduced latency to feed in a novel environment.
- Anxiolytic-like potential of diazepam is not affected by sex differences in mice, despite an increased sensitivity to restraint in the females.

References

1. Kundakovic, M., Rocks, D., 2022. Sex hormone fluctuation and increased female risk for depression and anxiety disorders: From clinical evidence to molecular mechanisms. Front Neuroendocrinol., this issue, https://doi.org/10.1016/j.yfrne.2022.101010.
2. Karp, N.A., Reavey, N., 2019. Sex bias in preclinical research and an exploration of how to change the status quo. Br J Pharmacol. 176(21), 4107-4118

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