OVERVIEW

Heitzmann et al. use natural variation in *Mus minutoides* female sex genotypes to ask how genotypic sex contributes to maternal care in this species. They find that females carrying the "sex reversal" X chromosome and Y (i.e. X*Y females) differ from other females in pup retrieval behavior, nest building, male-directed aggression, but not in other pup interactions. These behavioral results add a new layer of richness to this system, setting the stage for future inquiries into the genetic and neural bases of these behavioral differences and why this third genotype is maintained evolutionarily. The authors also perform some characterization of nonpeptidergic and dopaminergic populations across genotypic sexes, however small sample size and high variability limits the interpretability of these results.

COMPONENTS

Title: Clearly reflects article's contents.

Abstract: Concise and presents main findings. The dopaminergic results, however, I think are too limited (looking at data spread and sample size) to be interpreted as "likely" impacted by genotypic sex. More on this in the Results section.

Introduction: Research motivation and questions are well described with some exceptions. Regarding summarizing relevant prior work, I suggest making it explicitly clear which sex hormones have been compared between the genotypic sexes in *M. minutoides* specifically. Otherwise, it still seems open-ended as to whether the behavioral results are due to hormonal or genetic differences. Also, while the functional relationships between vasopressin and oxytocin and behavior are described, it is not abundantly clear what expectations are regarding cell population numbers and the *M. minutoides* genotypic sexes.

Materials and methods: There are several details that would improve the reproducibility of this work.

- Please provide time of experiments relative to light cycle (e.g. Zeitgeiber time).
- Please mention which, if any, criteria were used in pairing males and females. Also, please include female age at pairing and testing.
- Can you provide at average period between the "punctual observations" or maternal care strategy?
- Please mention what type of bedding was used during the nesting test. The bedding material would affect what kind of nest a mouse could build without using the cellulose nestlet.
- In the statistical analysis section, the following sentence seems to be missing a reference: "Both our models checked convergence recommendations ()."

Results: The behavioral results seem sound. The histological studies are quite limited in statistical power, which the authors openly state. While I can sympathize with the challenges of working with a study species that harder to procure and/or more variable than inbred strains, these data and provided analyses all fall below my threshold for identifying any "tendencies." I think the only sound conclusion to be made is that "there were no overall striking differences."

Discussion:

- In the section discussing chromosomal contributions to the X*Y phenotype, both the X* and the Y are discussed as potential sources of differences between X*Y females and the other female genotypes. Could X-inactivation escapees also play a role in the difference between X*Y females and the other female genotypes?
- I think the discussion of oxytocin and vasopressin could be made clearer regarding relationships between anatomy, gene expression, and behavior. Do the authors expect that, given the lack of striking difference in cells numbers, that there would also be a lack of gene expression/peptide difference? The authors also mention earlier that reproductive status can influence these systems; I would like to see that discussed here to contextualize their results.

Figures:

- The way the figures printed out for me, the axes labels for the Figure 2 and Figure 3 plots are very small. The genotype labels over brain images in Figure 2 are also very small.
- Figure S4 is missing “c” panel.
- Figures S8-10. These images appear to come a single, representative female of each genotype. That should be made clear in the figure legend. Also, only “a” is in the actual figures, “b” through “e” are only mentioned in the figure legend.

References: Seem fine, just need to address missing reference in Statistical Analysis section.