This revised manuscript has addressed most of my concerns with the initial version. There are a few minor outstanding issues that would be good to address.

Reviewer 2

Please provide much more detail in the methods. Here is a partial, but not exhaustive list:

1. How many generations were the mice bred in the lab after being caught in the wild?
2. Many more details on the husbandry of the animals is necessary. For example: what doyou feed them and how often, in what cages and what dimensions are they kept, is the room temperature controlled and what temperature, light cycle conditions, etc.
3. Are X* Y sisters and littermated of XX* ? Otherwise, there is a chance that differences inbehavior between the genotypes are related to other genetic differences between X*Y and XX* (and XX) due to population structure (in the wild or in the lab) rather than to the genotype of the sex chromosomes themselves. Please describe how the individuals used in the experiments were generated.

We added the information related to these 3 points lines 107-117. As for X* Y females, you are absolutely right ; however here, X*Y and XX* females are both littermated of X*Y or XX* females, while XX are littermated of XX or XX* females. In consequence, the three female genotypes can potentially be sisters. We thus eliminate population structure or maternal effects.

Point 1 is still not addressed in the manuscript.

Point 2 is mostly addressed but mentions mice are fed “bird seeds”. Is this supposed to be seeds for bird feed? Are they sunflower seeds or a mix?

4. Lines 119-120: How many pups are placed in the cage for tests of pup retrieval? Andhow was this normalized across females with litters of different sizes?

We used the entire litter which indeed varies between females, which is why we focused only on the first pup retrieved. It is described lines 131-134

5. The dimensions of the cages are not clear. Which one is the height?

It is standard nomenclature for dimensions so length x width x height

This is such an easy issue to fix, that I’m confused by the reluctance of the authors to provide more details to make it easier for readers.

6. Line 136: More details on the nestlet. What exactly is this compressed cellulose? Whatbrand? What dimensions and weight?

We added the informations lines 149-150(Serlab, D00009)

7. Line 140-141: I don't understand "If a nest was built without using cellulose". What elsecould it have been built with if you only provided cellulose as nesting material?

They can also use bedding, we clarified it.

8. The parental care strategy measurement is not clear due to insufficient details. Forexample, was this done in the home cage or a new cage? How long is each observation? Do you average all observations? Was the male ever separated from the female and added back for this assay or is this just observations in the cage where the female and male reproduced, without disturbing it. Also, regarding the assay and its interpretation, this measurement reflects the behavior of the male as much as that of the female. Are you confident you are measuring the "strategy" of the female rather than the interest of the male in the pups because random males are placed with females of all three genotypes?

More informations are provided in the specific section. All observations (every two-three days from day of parturition of the first litter to day 14) were made in home cage, so where male and female reproduce and without manipulation.

We talk about parental care strategy and not only female strategy in this experiment. Nevertheless, since the score attributed to the males relies on female behaviours : whether the female chases him (once the male goes nearby the female, she instantaneously attacks him) or includes him in the nest, we are confident that parental strategy relies mainly on the strategy of the female (it is the female behaviour towards the male that impacts the father's involvement). Even in bi-parental care, it depends mainly (only ?) of the choice of the female to allow the male to provide care. Therefore, we do have different strategies between females as the genotype is the only varying factor in these experiments.

It is an average population model because of repeated measure over time but not for all categories (we did not repeated measure when the score was 1 because we had to remove the father or he was already killed). Therefore we cannot make a standard ordinal model. In this model, we include all the data with information about repeated measures per individual and the corresponding score to estimate the average probability of falling into a class or under (see Touloumis et al, 2015 for further model explanation).

Other comments

9. Lines 156-157 and 401: I didn't understand why you cannot inject viruses into the brains of these mice nor euthanize mothers to perform analyses of their brains or other experiments.

We cannot for multiple reasons : probably the first one is our own personnal ethics about animal welfare, we do not feel comfortable about performing these approaches.
Second, It's a wild species that is hard to reproduce in laboratory and we cannot take the risk to kill mothers and the corresponding litters. Third, all experimentations such as optogenetic studies are performed on lab mouse, this system is not fit for pygmy mice that weight on average 8g with a size of 6cm. As for virus, similarly, it is based on lab mice and rats, we need stereotaxic coordinates of the brain which we do not have in our mice. It is the first study that investigates the brain of this wild mouse species.

I would still edit these sections so it doesn't say "as no experimentations leading to death can be made on mothers". It's not that these experiments can't be done, but rather that you are not comfortable doing them.

10. Lines 10-11: I think the authors mean that the sexually dimorphic nature of parental careis largely explained by differences in gonadal hormones between the sexes, but this is not clear.

Yes, we clarified this point in the abstract.

11. Lines 23-24 and 336-337: I'm not sure why the unique maternal care behavior of X*Yfemales is being labeled as a "third sexual phenotype". What is a "sexual phenotype"? And wouldn't the results of the authors actually be consistent with maternal behavior in these mice not being a "sexual phenotype" since all three of XX, XX* and XY* are females (gonadally and in terms of reproductive anatomy) so their (gonadal) sex doesn't differ, only their behavior?

It is not only the maternal behaviours that makes us talk about a third sexual phenotype. It is the combination of our present and previous results: agressive and anxiety related behaviours, life-history traits, bite strength etc.. (Saunders et al. 2014, 2016 ; Ginot et al. 2017). When we talk about sexual phenotype we need to distinguish between gonadal sex and other sexual traits such as gender (sexual characteristics/ differentiation of the brain). Gonadal sex is indeed either testis or ovaries ; but when it comes to other phenotypes : it is not dichotomous with either stereotypic male and stereotypic females. Sex is multiscale and gender for instance is not binary. The consistent divergence of patterns between the X*Y females and the other females and some similar traits between males and X*Y females allow us to talk about another female phenotype, therefore a third sexual phenotype.

I do not agree with the semantics of a "third sexual phenotype" since I find that it creates more confusion rather than clarity. But this is the authors' prerogative and it doesn't change the results or conclusions so they can use it if they wish.

12. Lines 30-31: Missing a comma after females. Also, "invest more notably as a primaryresult of an obligatory lactation" is not clear. A physiological result? An evolutionary consequence? There are mammals such as marmosets in which most of the parental care (except for lactation) is performed by males. Also, in birds, females often do more parental care than males, even though there's no lactation. Thus, higher maternal than paternal care is related to higher investment by females than males in offspring (including in making eggs and in preganancy), not necessarily a consequence of lactation itself.

We only talk about mammals here and lactation in mammals makes maternal care obligatory, so there already is a bias. But yes, you are right lactation does not necessarily mean female skewed parental care. Nevertheless, it is rare that fathers undertake more care than females as found in Marmosets. Usually, females invest more and they have no "choice" to invest because of lactation otherwise the offspring will die.

13. References such as Rice, 1984 are missing from the reference list.

It was present but misplaced, we rectified it.

14. Lines 75-78: I'm not convinced about calling high aggression, reduced anxiety andgreater bite force as "male-specific behaviors". I wouldn't, for example, call elevated height as a male-specific human trait, even though on average human males are taller than females. Please consider rephrasing.

The reduced anxiety behaviours and higher bite force of X*Y females are comparable to males but different from the other female genotypes (see Saunders et al., 2016, Ginot et al., 2017), therefore they show masculinized patterns. Concerning aggressive behaviours, even if they are observed in both sexes, these traits are mainly observed in males, they are sexually dimorphic in many diverse taxa. Furthermore, gonadal hormones and notably testosterone act perinatally to induce masculinization of neural pathways involved in sexually dimorphic behaviours including aggression.

Comments 15, 19 & 20:

15. Lines 78-79: I don't think there's enough evidence to say that the Y chromosome masculinizes neural circuits in X*Y M. minutoides. It could be a lack of a second X chromosome or X* masculinizing them when there is a Y chromosome (i.e. an epistatic effect).

Indeed, it is an hypothesis based on the study of Gatewood et al., 2006 where they made the assumptions that Sry was a strong candidate for nest building inhibition as sex reversed females XY without Sry built good quality nest. You are absolutely right though, it can be an X dosage imbalance females being heterogametic even if results of Gatewood et al, strongly suggest that nest building is not impacted by dosage imbalance.

19. Lines 323-327: I don't understand this very long and convoluted sentence. Also, there isno evidence from this study that "masculinization" of X*Y females is caused by Sry. See comment 15.

We rectified the sentence and yes, you are right, it was an hypothesis based on previous findings (Gatewood et al., 2006), we clarified it.

20. Lines 329-332: Related to point 14 above, how is this evidence of masculinization or of a"hyperfeminine" trait? I would consider rephrasing these sections.

We changed "hyperfeminized" to 'feminized'. We agree it is more appropriate, we used hyper-feminized because they have a higher reproductive success than XX and XX* females : higher ovulation rate, greater litter size, precocious sexual maturity but considering maternal behaviours, it is not relevant. As for masculinization we talk about masculinized trait because the pattern observerd in X* Y females is similar to that of males.

16. Line 245: "on average" instead of "in average".

Indeed, it is now changed.

17. Lines 278-279 and 384-385: Because you did not directly compare the neuroanatomy ofAVPV between M. minutoides and M. musculus, the conclusion that “there were no differences in the neuronatomy of the AVPV in comparison to M. musculus” is a bit of a stretch. Please rephrase.

The neural structures, such as the paraventricular nucleus (same shape and neuronal populations ; expression/ distribution of oxytocin and vasopressin in parvocellular and magnocellular neurons) are overall similar between musculus and minutoides which we explain right after lines 279-282. We added references lines 284-285 and lines 297-300.

18. Lines 302-303: No direct comparison of aggressiveness was done between pre and postparturition, so this conclusion is not warranted.

Increases was indeed not appropriate, we changed it.

21. Lines 347-350: Related to point 15, this could also be due to epistasis between X* and Y?

It is a very good question and we cannot rule out an epistasis interaction, we included it in the X* chromosome effect but we clarified it along with the hypotheses on the rôle of each sex chromosome in the paragraph (line 354-401).

For instance and on that point, assuming we confirm the pattern of Th between females as we found also on RT-qPCR (see comment reviewer 3), one could hypothesize an epistasis interaction between the X* and Y on the dopaminergic system :1- we know that individuals carrying a single X chromosome have usually more dopamine, 2- Sry positively modulate dopamine through Th and 3- The AVPV (based on males *musculus*) is a rare region of the brain where Th is greater in females.
One could therefore assume an adaptive positive regulation of X* in interaction with Sry on the Y chromosome.

22. Line 397: What is a species with “precious” status?

Thank you for this comment, indeed, precious is not relevant here. We meant that some individuals are rare, it can be hard to breed them especially XX females. We changed it