Abolition of the sex difference in Ngn3 by estradiol is depending on sex chromosome complement

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INTRODUCTION

A growing body of evidences indicates that some sexually dimorphic traits cannot be solely explained as a result of gonadal steroid action during the critical period of brain masculinization (E18-PN10).

Neurogenin 3 (Ngn3), a gene located in mouse chromosome 10 (MGI:893591), is involved in neuritogenesis and morphological differentiation of hippocampal neurons (Salama-Cohen et al., 2006).

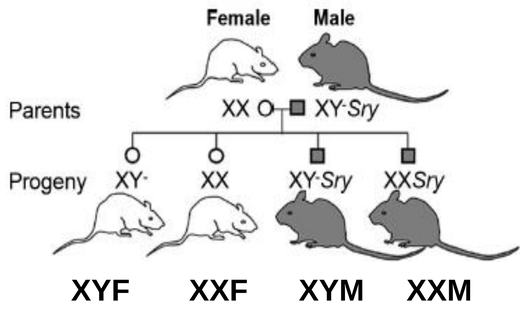
Recent works from our laboratory have shown the existence of sex difference in the neuritogenic transcription factor Ngn3 in hypothalamic neurons before brain masculization. Moreover 17β-estradiol (E2) abolishes this sex difference (Scerbo et al., 2014).

The sex difference in Ngn3 in hypothalamic neurons is depending on sex chromosome complement (Scerbo et al., 2014).

In order to study if cell-autonomous actions of sex chromosomes are involved in the effect of E2 on Ngn3, we evaluated Ngn3 mRNA in neuronal cultures.

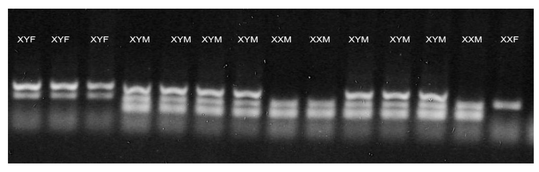
MATERIALS AND METHODS

- The embryos used for this study were obtained from MF1 wild type and four core genotypes (FCG) mice (Arnold and Chen, 2009).



- Neuronal hypothalamic cultures of E15 embryos were performed segregated by sex and genotype.

- RNA were extracted and cDNA obtained by reverse transcription. Real-time PCRs were performed using the TaqMan PCR Master Mix. Relative mRNA expression level was calculated using the ΔΔCT method.



Genotype was determined by PCR identifying the presence of Y long arm gene family *Ssty* and the *Sry*. *Myogenin* gene was used as a positive control.

RESULTS

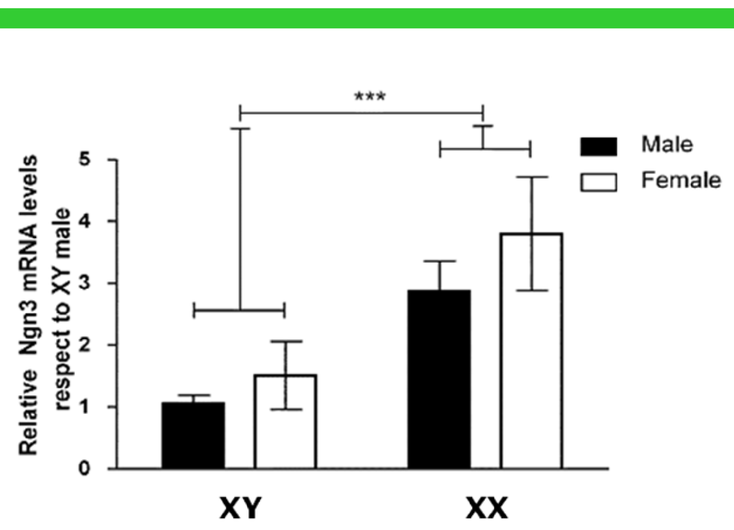


Figure 2: Sex differences in Ngn3 expression in primary hypothalamic neurons were determined by sex chromosomes.
Ngn3 mRNA levels in FCG model. Two-way ANOVA revealed no effect of gonadal sex but a significant effect of sex chromosome complement ($F_{1,28}$=12.60; p<0.001).
*** p<0.001.

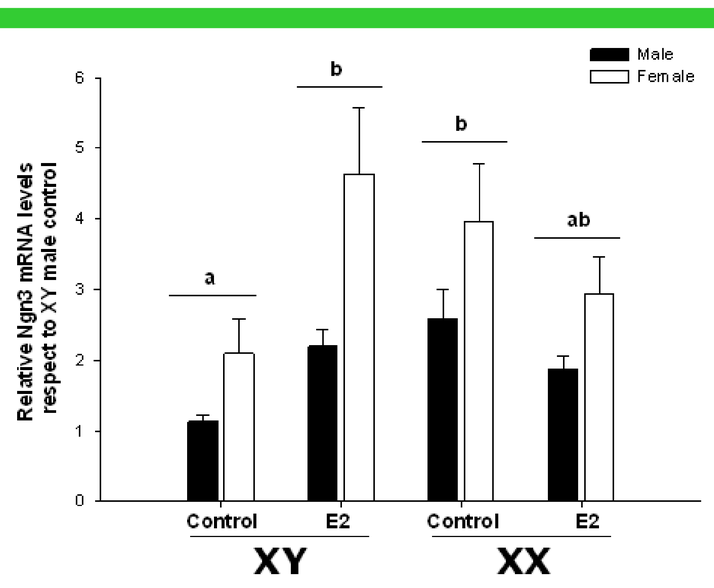


Figure 3: Abolition of the sex difference in Ngn3 expression by estradiol
Ngn3 mRNA levels in FCG model. Two-way ANOVA revealed a significant interaction of genotype and estradiol treatment ($F_{(1,45)}$=9.2005; p<0.004). Different letters indicate p<0.001.

CONCLUSIONS

Sex chromosome complement determines sex differences in Ngn3 expression: cells carrying different sex chromosome gene(s) showed different Ngn3 mRNA levels (XY<XX). In addition, the E2-treatment resulted in a significant increase in the expression of Ngn3 only in cultures of neurons carrying XY chromosomes (p<0.001), irrespectively of the gonadal type (XYM and XYF). E2 did not significantly affect Ngn3 mRNA levels in XX cultures.

In summary, these findings indicate that the expression of the autosomal Ngn3 gene must be downstream the expression of X or Y genes that results from the inherent sex difference in the number (two copies of X) and/or type (presence of Y) of sex chromosome and that sex chromosome complement mediates both cell-autonomous and hormonal actions on Ngn3 in the hypothalamus.

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