EXPRESSION OF KEY STEROIDOGENIC ENZYMES IN DEVELOPING BRAIN: HORMONAL COMPENSATION OF SEX CHROMOSOMES-INDUCED SEX DIFFERENCES

Cisternas CD, Tome K, Cambiasso MJ.

Instituto Ferreyra, INIMEC-CONICET, Universidad Nacional de Córdoba. Córdoba-Argentina jcambiasso@immf.uncor.edu

INTRODUCTION

Developing brain of mammals is organized by gonadal steroids during the critical period of sexual differentiation (E18-PN10). The regulatory role of neurosteroids in the early brain is unclear. It is known that 17-β-estradiol (E2) is produced within the brain itself primarily due to local aromatization of gonadal testosterone and also due to *de novo* synthesis from cholesterol.

Little is known about the circulating and local levels of steroids in the embryonic brain. Thus, the use of animal models that dissociate the effect of gonadal sex from sex chromosomes heritage facilitates the study of organizational actions of gonadal steroids and neurosteroids in each sex.

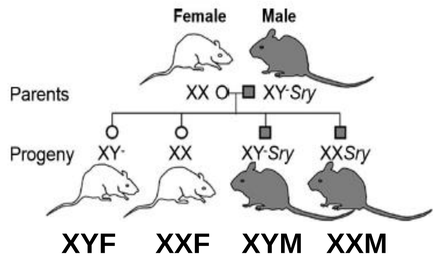
OBJECTIVE

- Evaluate the expression of key steroidogenic enzymes and the mitochondrial cholesterol transporter Star in the brain of the "four core genotypes" mouse model, in which the effect of gonadal sex (testes vs. ovaries) and sex chromosome complement (XX vs. XY) are dissociated, allowing comparisons between XX and XY females as well as in XX and XY males.

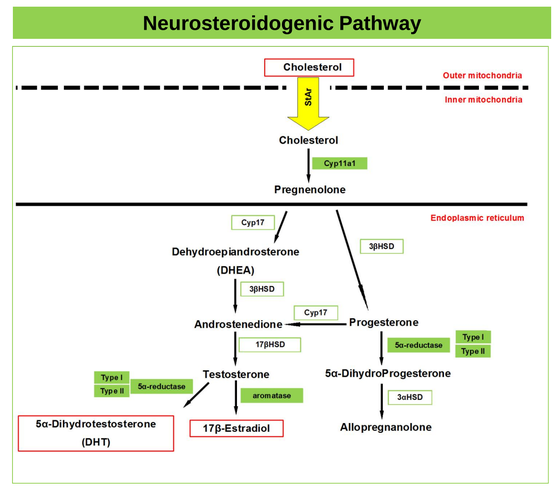
MATERIALS AND METHODS

- Punches of Stria Terminalis, Anterior Amygdaloid Area and Amygdala were obtained from E16 mice brain segregated by genotype.
- Neuronal cultures of amygdala of E15 embryos were performed segregated by sex and genotype. Cell treatments: Cultures were treated with 17β-Estradiol (10^{-10} M) or Dihydrotestosterone (10^{-10} M) at 3 DIV.
- RNA were extracted and cDNA obtained by reverse transcription. Real-time PCRs were performed using the Power Sybr Green PCR Master Mix.
- We evaluated the expression of mRNA for star (steroidogenic acute regulatory protein), cyp11a1 (cytochrome P450-side chain cleavage), cyp19a1 (cytochrome P450-aromatase), srd5a1 and srd5a2 (5 alpha reductases I and II).

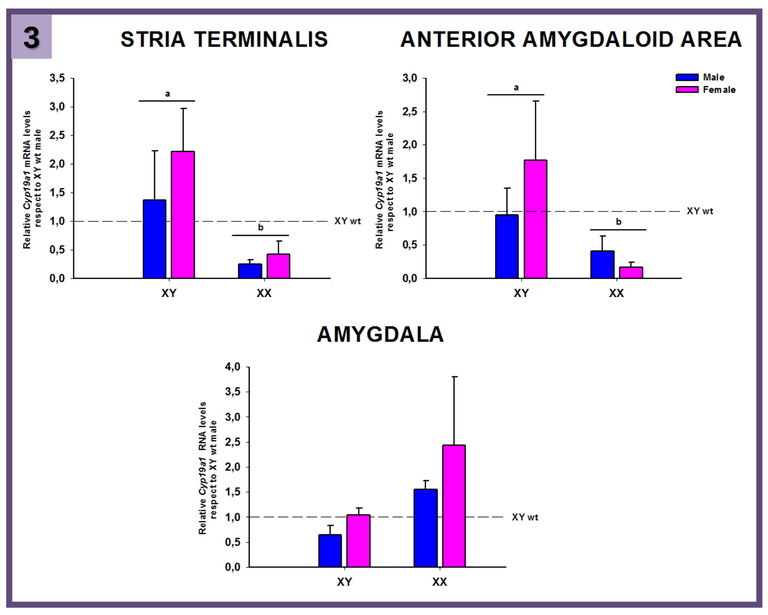
Four Core Genotype Mice Model

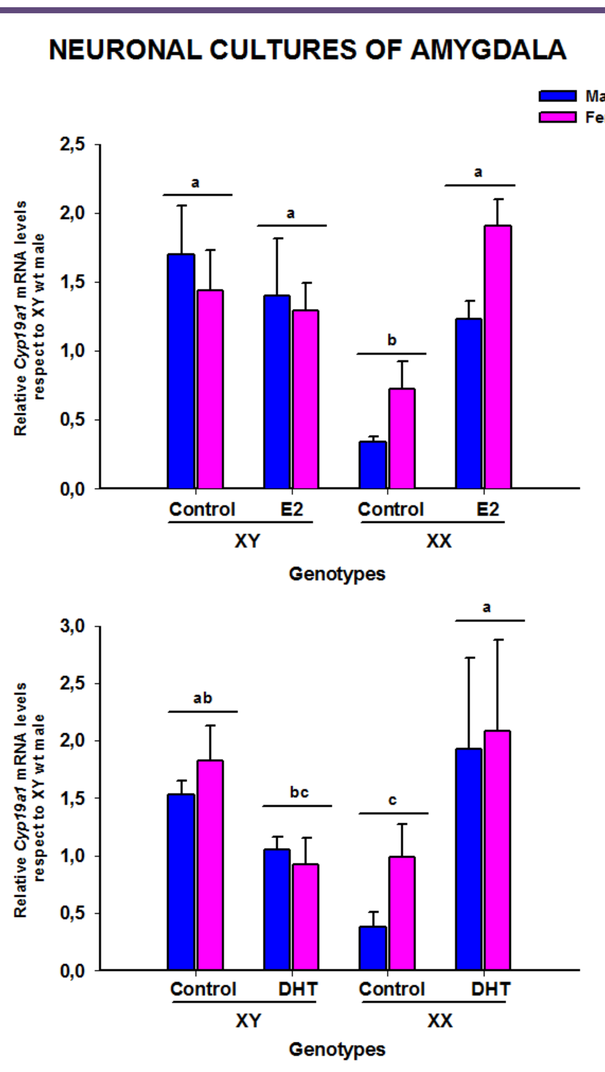


Neurosteroidogenic Pathway



RESULTS

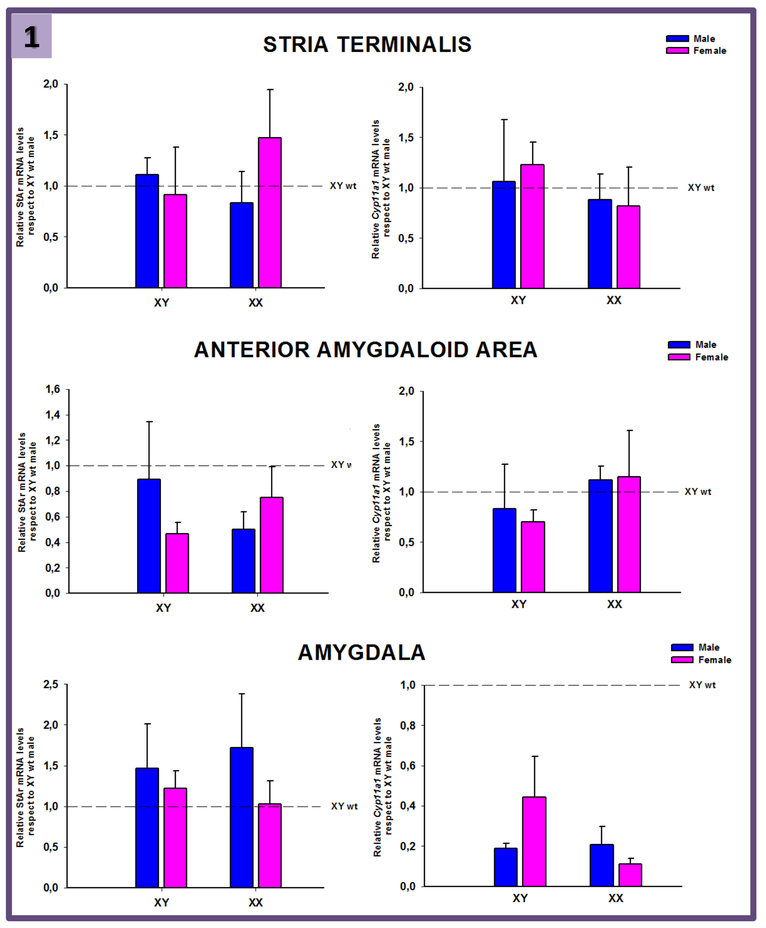


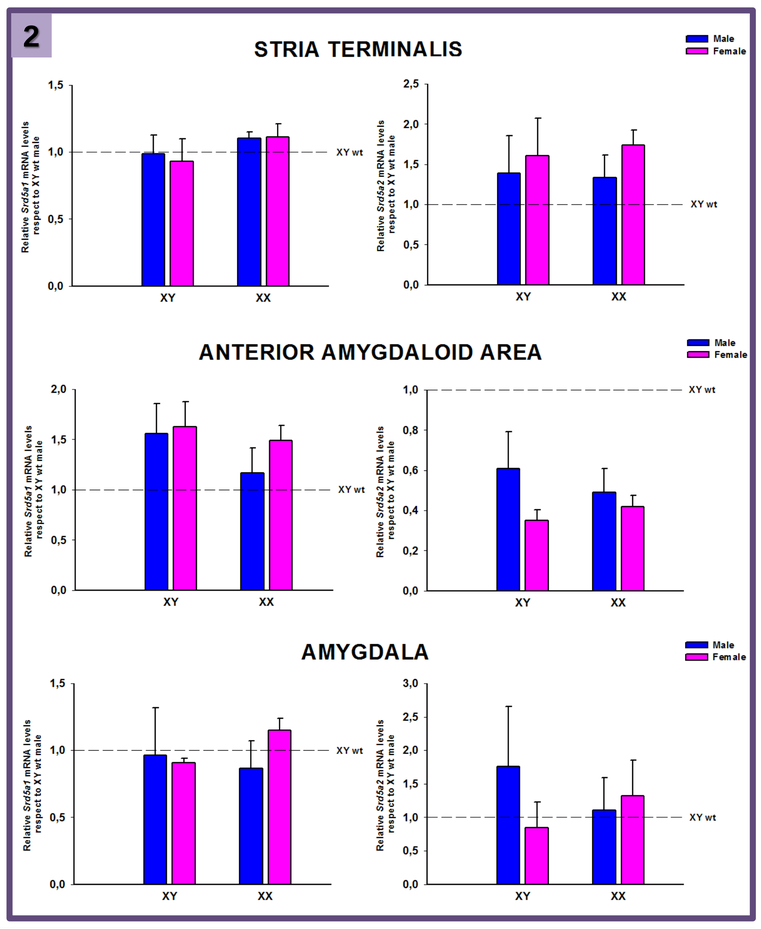


Aromatase mRNA levels in amygdaloid neurons at 3 DIV. ANOVA revealed a significant effect of interaction between hormonal treatment and genotype ($p<0.05$). XX cultures treated with 17β-Estradiol or Dihydrotestosterone (DHT) showed similar expression levels in aromatase mRNA than XY control cultures. Different letters indicates significant differences with $p<0.05$

RESULTS

Relative quantification of mRNA for: 1) Star and Cyp11a1; 2) 5α-reductase type I and II (Srd5a1 and Srd5a2); 3) aromatase (Cyp19a1) in punch of Stria Terminalis, Anterior Amygdaloid Area and Amygdala of E16 mouse brain. (n=4). Two-way ANOVA revealed a different expression in Cyp19a1 between XY and XX brain independently of gonadal sex. Different letters indicates significant differences with $p<0.05$





CONCLUSIONS

- Our results show that among the enzymes and the cholesterol transporter analyzed only the brain expression of aromatase is determined by sex chromosomes at embryonic day 16.
- The *in vitro* treatment with 17β-estradiol or DHT abolished these sex chromosome effect suggesting that gonadal steroids could compensate *in vivo* the differences caused by sex chromosomes in aromatase expression in the brain.
- These results suggest that sex chromosomes and steroid hormones interact to produce and reduce the sex differences observed in phenotype.

FUNDING: SECyT-UNC; MINCyT-CBA; FONCyT