Sex differences in active DNA demethylation machinery during the critical period of brain masculinization

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INTRODUCTION

In mammals, perinatal peaks in gonadal testosterone organize a sex-typical neural circuitry during sensitive periods of development and a growing body of evidence suggest that epigenetic mechanisms are implicated. Some of the hormonal effects determine stable, sex-specific patterns of gene expression in neurons leading to the differentiation of neurochemical phenotypes relevant for the display of innate social behaviors in adulthood. We recently found that a neonatal inhibition of DNA methylation or demethylation reduces or eliminates sex differences in neurochemical phenotypes found in hypothalamic and preoptic regions of the mouse brain (Cisternas et al., 2020a). In line with this, we reported dynamic changes and sex differences in DNA methylation during the first postnatal week (Cisternas et al., 2020b).

Aim

Assess sex differences and E2 regulation of the DNA demethylation mechanism during and after the critical period of brain sex differentiation.

Hypothesis

Estradiol regulates sexual differentiation during development through DNA methylation/demethylation mechanisms.

MATERIALS AND METHODS

Tissue samples from P7, and P20 male and female mice were micropunched from POA (Preoptic Area), PFC (Prefrontal Cortex) and PVN (Paraventricular Nucleus) using a cryostat at -20°C. Total RNA was extracted using Trizol (Invitrogen) at 4°C following manufacturer instructions and quantified with Nanodrop at 260nm. Total RNA was treated with DNaseI (Fermentas) and 1 ug of RNA was reverse transcribed using M-MLVL enzyme and random primers (Biodymamics). The expression of Gadd45a and Gadd45b, TDG, Tet1, Tet2, Tet3 and the oxytocin receptor (OTR) was quantified by qPCR using 2X Sybr Green Master Mix (Roche) in a total volume of 10 µl. The amplification was carried out in the StepOne™ Real Time PCR System (Applied Biosystems).

RESULTS

PFC

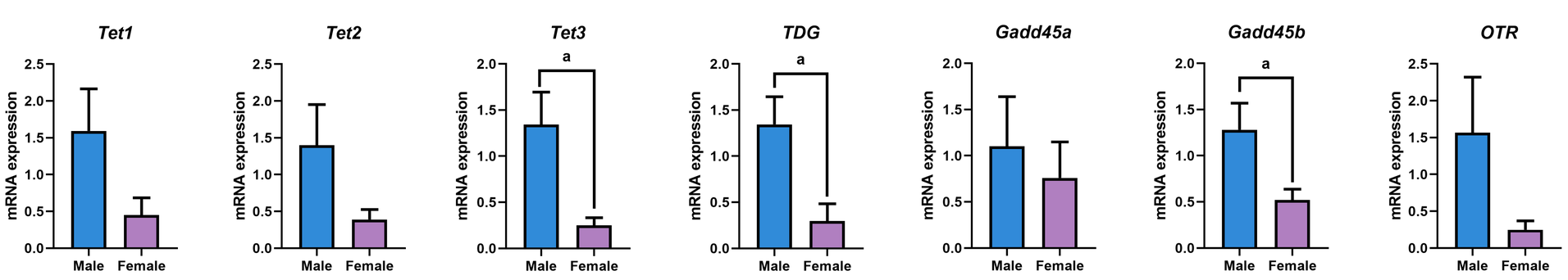

Figure 1. *Tet1*, *Tet2*, *Tet3*, *TGD*, *Gadd45a*, *Gadd45b* and OTR mRNA expression in the Prefrontal Cortex (PFC) of P7 mice. Symbol "a" indicates significant differences with p< 0,05. n= 3 - 4

PVN

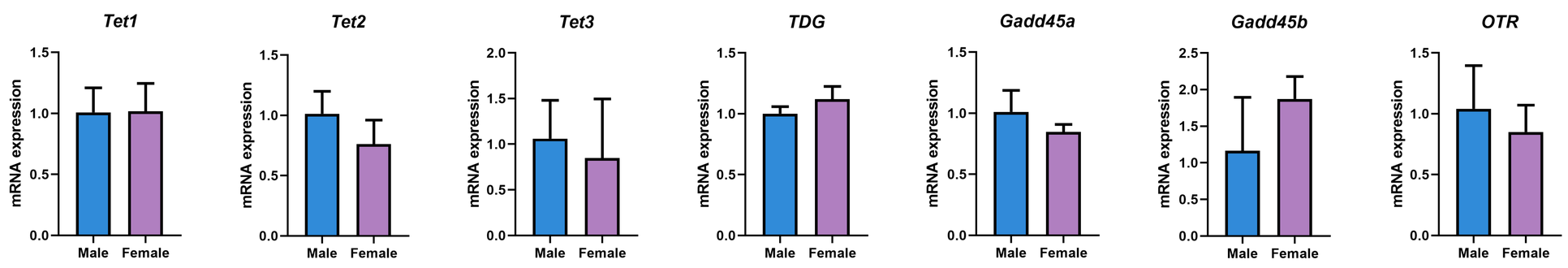

Figure 2. *Tet1*, *Tet2*, *Tet3*, *TGD*, *Gadd45a*, *Gadd45b* and OTR mRNA expression in the Paraventricular Nucleus (PVN) of P7 mice. n=4

POA

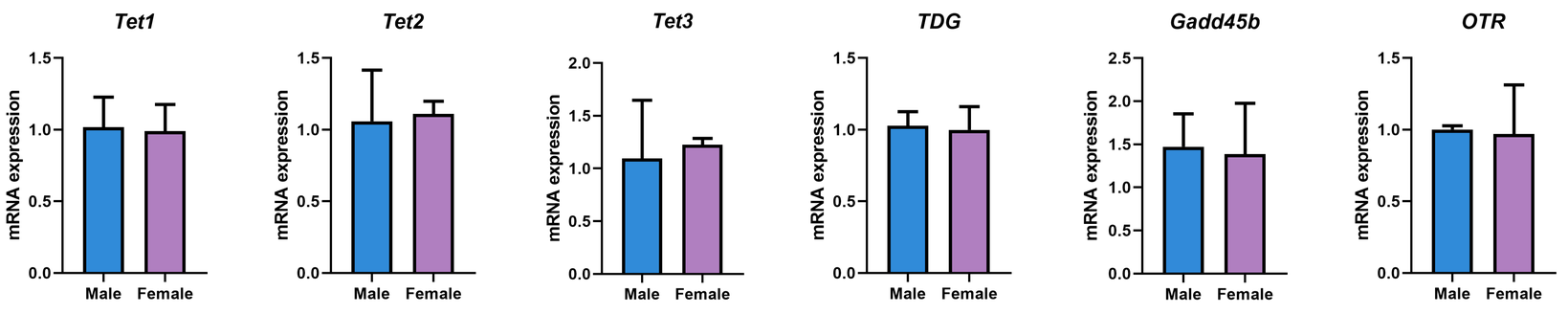

Figure 3. *Tet1*, *Tet2*, *Tet3*, *TGD*, *Gadd45b* and OTR mRNA expression in the Preoptic Area (POA) of P7 mice. n=4

CONCLUSIONS

In PFC, we found sex differences in Tet3, TDG and Gad45b expression ($p<0.05$) and a trend for higher expression of OTR in males ($p=0.06$) at P7 suggesting higher DNA demethylation in males during the critical period of sexual differentiation. In PVN and POA we did not find sex differences in mRNA expressions at P7. There were no differences at P20 in any region.

Overall, these results suggest that a sex-specific pattern of active DNA demethylation machinery could underline the organizational effects of hormones.

REFERENCES

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ACKNOWLEDGEMENTS

CONICET AGENCIA International Brain IBRO Research Organization ISN International Society for Neurochemistry