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Research Report

Sex differences in Gadd45b expression and methylation in the developing rodent amygdala

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ABSTRACT

Precise spatiotemporal epigenetic regulation of the genome facilitates species-typical development; sexual differentiation of the brain by gonadal hormones and sex chromosomes causes extensive epigenetic reprogramming of many cells in the body, including the brain, and may indirectly predispose males and females to different psychiatric conditions. We and others have demonstrated sex differences in DNA methylation, as well as in the enzymes that form, or 'write', this epigenetic modification. However, while a growing body of evidence suggests that DNA methylation undergoes rapid turnover and is dynamically regulated *in vivo*, to our knowledge no studies have been done investigating whether sex differences exist in the epigenetic 'erasers' during postnatal development. Here we report sex differences in the expression of growth arrest and DNA damage inducible factor β (Gadd45b), but not family members α (a) or γ (g), in the neonatal and juvenile rodent amygdala.

1. Introduction

The developing mammalian brain is uniquely sensitive to environmental and hormonal cues; during so-called 'sensitive periods', extensive epigenetic programming and reprogramming is occurring (Auger et al., 2011a, 2011b; Roth and David Sweatt, 2010; Weaver et al., 2004). Abnormalities in the epigenome acquired during development can cause long-lasting and adverse effects on such far-reaching behavioral and physiological traits as memory (Korosi et al., 2012), parental behavior (Champagne et al., 2006), sexual differentiation (Auger and Jessen, 2009; Matsuda et al., 2011; Nugent et al., 2015), drug-seeking behavior (Massart et al., 2015), and are furthermore linked to psychiatric dysfunction (Bagot et al., 2014; Labonté et al., 2013; McGowan et al., 2009). In fact, drugs directed at epigenetic factors are a proposed target in the treatment of psychiatric disorders (Szyf, 2015). Interestingly, in addition to altered programming of the neural epigenome, biological sex is a highly salient risk factor in the development of psychiatric disorders (Chase et al., 2015, reviewed in Kigar and Auger (2013)). This makes it a priority to understand the basic biological signaling mechanisms at play in controlling the epigenome during sexual differentiation.

We and others have previously demonstrated sex differences in the abundance of epigenetic modifications such as 5-methylcytosine (5mC) (Kurian et al., 2010; Nugent et al., 2015) and histone acetylation or methylation (Tsai et al., 2009). We have also shown that there are sex differences in the expression of epigenetic factors that increase or decrease gene transcription; specifically, males have higher levels of co-activators such as steroid receptor coactivator-1 (Src-1) (Auger et al., 2000) and cyclic-AMP response element-binding protein (CBP) (Auger et al., 2002), whereas females have higher levels of co-repressors such as methyl cytosine binding protein 2 (MeCP2) (Kurian et al., 2007) and nuclear receptor corepressor (NCoR) (Jessen et al., 2010). Finally, sex differences have been observed in the expression or activity of enzymes that catalyze formation of epigenetic modifications, including DNA methyltransferase 3a (Dnmt3a) (Kolodkin and Auger, 2011), Dnmt1 (Nugent et al., 2015), and several histone deacetylases (Xu et al., 2008a, 2008b).

While DNA methylation was previously thought to be a static and/or permanent epigenetic modification (Wu and Zhang, 2010), a growing body of evidence suggests it undergoes rapid turnover and is dynamically regulated *in vivo* (Auger et al., 2011a, 2011b; Feng et al., 2010; Ma et al., 2009). Briefly, ten-eleven translocation (Tet) enzymes are able to catalyze conversion of 5mC into 5-hydroxymethylcytosine (5hmC); 5hmC will eventually be converted to a uracil analog, resulting in a DNA base pair mismatch that will

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be excised. Importantly, the growth arrest and DNA damage inducible factor (Gadd45) β (b), α (a) and γ (g) family of proteins appear to participate in DNA demethylation through an as-yet unidentified mechanism (Niehrs and Schäfer, 2012); in particular, Gadd45b is required for stimulus-induced DNA demethylation (Ma et al., 2009).

We have recently demonstrated a role for Gadd45b in dampening juvenile male rats' drive to engage in 'rough and tumble play' (Kigar et al., 2015), suggesting it may be an important risk factor in the development of abnormal social behaviors. We were specifically looking at the function of Gadd45b within the developing amygdala – a region of the brain critically important in the formation of socioemotional behaviors (LeDoux, 2007; Phelps and LeDoux, 2005; Shaw et al., 2004). While sex differences in Gadd45b mRNA expression have been observed in the adult prefrontal cortex (Blaze and Roth, 2013), it is unknown whether sex differences exist in its expression in steroid hormone-responsive brain regions, e.g. the amygdala and hypothalamus. Here we address this knowledge gap by examining the expression of the Gadd45 protein family at two important developmental time points – twenty-four hours after birth and the beginning of the juvenile social play period.

2. Results

2.1. mRNA sex differences in demethylase factors at postnatal day 1 (P1)

Sex differences in the expression of Gadd45b, but not related family members Gadd45a and Gadd45g, were found in the P1 amygdala, where females had higher levels of Gadd45b mRNA than males: [Gadd45b (female: 0.6337 ± 0.05103, $N=9$; male: 0.4061 ± 0.02500, $N=8$. $p=0.0016$); Gadd45a (female: 0.5947 ± 0.03712, $N=8$; male: 0.5194 ± 0.04059, $N=9$. $p=0.1947$); Gadd45g (female: 0.5548 ± 0.1090, $N=10$; male: 0.5148 ± 0.1017, $N=8$. $p=0.7958$)] (Fig. 1A). We observed no sex differences in Gadd45 family members in the P1 hypothalamus: [Gadd45b (female: 0.6698 ± 0.05339, $N=8$; male: 0.5863 ± 0.03181, $N=9$. $p=0.1877$); Gadd45a (female: 0.5775 ± 0.07341, $N=8$; male: 0.4809 ± 0.04452, $N=10$. $p=0.2566$); Gadd45g (female: 0.6181 ± 0.06382, $N=8$; male: 0.5893 ± 0.04411, $N=9$. $p=0.7108$)] (Fig. 1B).

2.2. Effect of hormone treatment on Gadd45 expression in females

Neonatal females were injected subcutaneously with hormone or vehicle on P0 and P1 before sacrifice on P2. There was a significant overall effect of hormone treatment on the expression of Gadd45b mRNA in females, where dihydrotestosterone (DHT)-treated females showed less expression than oil-treated females (one-way ANOVA; $F_{(3,27)}=4.130$, $p=0.0170$. Tukey's *post-hoc*; $q=4.873$) (Fig. 2A). We observed no effects of hormones on remaining family members Gadd45a or Gadd45g: [Gadd45a (oil: 0.4269 ± 0.04353, $N=8$; testosterone: 0.4621 ± 0.05316, $N=7$; estrogen: 0.4041 ± 0.06577, $N=6$. dihydrotestosterone: 0.3387 ± 0.02716, $N=7$. $p=0.3371$); Gadd45g (oil: 0.4424 ± 0.09015, $N=6$; testosterone: 0.4833 ± 0.08918, $N=7$; estrogen: 0.4458 ± 0.1031, $N=6$. dihydrotestosterone: 0.4555 ± 0.1221, $N=7$. $p=0.9917$)] (Fig. 2B and C).

2.3. mRNA sex differences in Gadd45 family members at postnatal day 25 (P25)

Sex differences in the expression of Gadd45b, but not related family members Gadd45a and Gadd45g, were also found in the P25 amygdala, where females had higher levels of Gadd45b mRNA than males: [Gadd45b (female: 0.6490 ± 0.04799, $N=10$; male: 0.4676 ± 0.04679, $N=8$. $p=0.0170$); Gadd45a (female: 0.5579 ± 0.04441, $N=10$; male: 0.6194 ± 0.06860, $N=9$. $p=0.4535$); Gadd45g (female: 0.5682 ± 0.08165, $N=10$; male: 0.6458 ± 0.02790, $N=8$. $p=0.4268$)] (Fig. 3A). We observed no sex differences in Gadd45 family members in the P25 hypothalamus: [Gadd45b (female: 0.6683 ± 0.03507, $N=11$; male: 0.6340 ± 0.03228, $N=8$. $p=0.4976$); Gadd45a (female: 0.6206 ± 0.03230, $N=11$; male: 0.5424 ± 0.01264, $N=7$. $p=0.0820$); Gadd45g (female: 0.5959 ± 0.02852, $N=10$; male: 0.6353 ± 0.03555, $N=9$. $p=0.3961$)] (Fig. 3B).

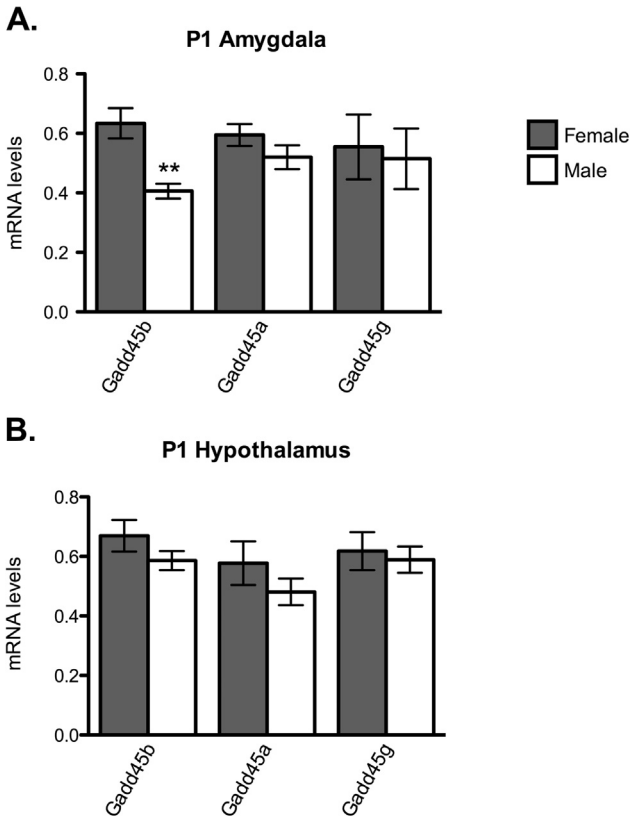

Fig. 1. Sex differences in the mRNA expression of Gadd45 family members Gadd45b, Gadd45a, and Gadd45g at postnatal day 1 (P1) in two brain regions A. amygdala and B. hypothalamus known to be steroid hormone-responsive during early neonatal development. $^{**}p < .01$.

2.4. Methylation changes in the Gadd45b promoter

We used a methylation sensitive restriction enzyme (MSRE) assay to examine relative amounts of DNA methylation at an estrogen receptor α (ERα) response element (ERE) in the P25 amygdala Gadd45b promoter. Doing so, we found sex differences in methylation at an AciI (CCGC) cut site, where males had more than females: Gadd45b (female: 0.3972 ± 0.04209, $N=10$; male: 0.6356 ± 0.1068, $N=9$. $p=0.0453$) (Fig. 4).

3. Discussion

Herein we present data describing neurodevelopmental sex differences in the mRNA expression of Gadd45b, a protein involved in DNA demethylation (Ma et al., 2009). Of the three Gadd45 family members, Gadd45b alone exhibited hormone responsivity in the amygdala, but not the hypothalamus (Figs. 1 and 3). Specifically, females expressed greater levels of Gadd45b mRNA neonatally (Fig. 1A) and this effect was also present during the juvenile period (Fig. 3A). The reduced mRNA expression observed in