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Research Article

BRAINSTEM EXPRESSION OF SLC6A4, HTR2C, NGF, BDNF, TRKA^{NGF}, TRKB^{BDNF} AND P75^{NTR} FOLLOWING PATERNAL ALCOHOL EXPOSURE IN THE MALE MOUSE

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We previously showed in the mouse that paternal preconception alcohol exposure (PPAE) affects alcohol sensitivity by analyzing postnatal alcohol preference in the offspring. In this mouse study by using the same animals of the previous investigation we aimed at examining whether or not PPAE may disrupt the epigenetic regulation of postnatal alcohol sensitivity in the offspring by investigating pathways regulating mood, emotion, serotonergic tone and neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). We analyzed the brainstem gene expression of serotonin transporter Solute Carrier Family 6 Member4 (SLC6A4), 5-Hydroxytryptamine Receptor 2C (HTR2C) binding the neurotransmitter serotonin, and NGF, BDNF and their tropomyosin receptor kinase A (TrkA^{NGF}) and B (TrKB^{BDNF}) (high-affinity NGF and BDNF receptors) and p75^{NTR} (low-affinity, pan-neurotrophins receptor) in adult offspring that underwent or not postnatal alcohol exposure. We found SLC6A4 elevation and decreased HTR2C in the offspring of chronic alcohol-exposed sires. We also disclosed p75^{NTR} elevation in the offspring of chronically exposed sires as well as postnatal sensitization to low alcohol doses in the offspring of chronically exposed sires for both TrKB^{BDNF} and BDNF. In our PPAE mouse model, where genotype effects can be carefully measured, we observed that the sires' exposure to alcohol before mating might disrupt the sensitivity to the serotonergic/neurotrophic-associated effects of alcohol influencing the postnatal alcohol preference in the offspring. **Biomed Rev 2020; 31: 75-89**

Keywords: SLC6A4; BDNF; NGF; TrkA^{NGF}; TrKB^{BDNF}; HTR2C; p75^{NTR}; Brainstem; Epigenetic; Pre-conceptual Paternal Alcohol Drinking

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1. INTRODUCTION

Differently to the abundant attention given to the influence that maternal factors have on the outcome of pregnancy exposed to alcohol (FASD, fetal alcohol spectrum disorders), minor attention has been given about the impact of paternal preconception alcohol exposure (PPAE) role on the offspring neurobehavioral and developmental features (1-3) even though both PPAE and FASD may induce similar consequences and very often they are overlapping (2). In particular, FASD are a group of conditions that can occur in a person whose mother drank alcohol during pregnancy. Problems may involve an abnormal appearance, low body weight, low intelligence, short height, small head size, behavior changes, poor coordination, and/or hearing or seeing deficits (4–9). Fetal alcohol spectrum disorders, and under certain aspects PPAE, have a very broad phenotype that is further complicated by high rates of comorbidity - over 400 disease conditions have been reported to co-occur in people with FASD. Further, it was recently estimated that approximately one out of every 13 prenatally alcohol exposed infants will have FASD, which results in approximately 630 000 infants being born with FASD each year in the world (10–13). Given that FASD is a lifelong disability, it is estimated that more than 11 million individuals between 0 and 18 years of age, and 25 million individuals between 0–40 years of age, have FASD in the general population worldwide (10–13).

As for alcoholism (14), also recognized as alcohol use disorder (AUD), it is a wide term for any drinking of alcohol resulting in mental or physical health problems (15–22). The World Health Organization estimates that as of 2010 there were 208 million people with alcoholism worldwide (4.1% of the population over 15 ears of age) (23–25). Despite its huge impact on society, AUD has few reliable pharmacological treatments and a high frequency of relapse (26–31). Though the AUD heritability is supposed to be □50% among men (32), the genetic basis for this disease is poorly understood despite significant scientific investments. Emerging data from several converging fields have strengthened the idea that inheritance of acquired characteristics, (i.e. epigenetic inheritance) is a further route of transmission to established genetic inheritance (33–35). In animal model studies in which genetics and environment can be meticulously controlled, it is now recognized that environmental changes including stress, unbalanced diet and endocrine disruptors may produce phenotypic (without genotypic) perturbations in subsequent generations not exposed to any specific challenge (36). A growing literature is available on the transgenerational effects of alcohol abuse. Indeed, prenatal exposure to alcohol was associated with transgenerational effects on hypothalamic proopiomelanocortin gene expression that was inherited through the male germ line (37). Paternal preconception ethanol exposure elicited developmental disruptions including altered organ weights (38, 39), changes in cortical layers (40) and abnormal testosterone levels (41). PPAE in rodents also induced numerous behavioral abnormalities (2), changes that did not appear to be related to stress and/or undernutrition associated with alcohol exposure.

We have previously examined in the mouse brain (1) that PPAE may disrupt nerve growth factor (NGF) and/or brain-derived neurotrophic factor (BDNF), neuropeptides of the neurotrophins family playing crucial roles in the growth, development and survival of nerve cells, also in the pathobiology of cardiometabolic and neuropsychiatric diseases (42–58). We found that PPAE affected NGF levels in frontal cortex, striatum, olfactory lobes, hippocampus and hypothalamus. Likewise, BDNF alterations in frontal cortex, striatum and olfactory lobes were found. In this previous study we also investigated whether or not PPAE could affect alcohol preference/rewarding properties in the male offspring (1). Furthermore, PPAE produced a higher susceptibility to the alcohol rewarding effects mostly apparent at the lower concentration (0.5 g/kg) that was ineffective in non-PPAE offspring. Besides, higher ethanol concentrations (1.5 g/kg) produced an aversive response in PPAE animals and a significant preference in non-PPAE offspring. We disclosed also that PPAE affected $TrkA^{NGF}$ (the high affinity NGF receptor) in the hippocampus and $p75^{NTR}$ (the low affinity neurotrophins receptor) in the frontal cortex. Intriguingly, PPAE disrupted the expression of the dopamine active transporter (DAT) in the olfactory lobes in offspring exposed postnatally to 0.5 g/kg of ethanol while no differences were found on the brain dopamine receptors D1/D2. In this previous study we speculated that, through epigenetic pathways, PPAE may alter NGF and BDNF expression in the mouse brain affecting also alcohol intake preference in the male offspring because of crucial modifications in the feeling of well-being and in the rewarding system (1, 59). To date, overlapping changes in brain neurotrophins were also found in the offspring of FASD mouse models (60–63).

The PPAE investigations presented above (1) led us to postulate that the mechanisms controlling the alcohol drinking behavior and the neurobiological sensitivity to alcohol may be due to disrupted epigenetic regulation of pathways regulating mood, emotion and serotonergic states potentially involved in depression and sickness behavior throughout also changes in

the expression of neurotrophins as NGF and BDNF. To validate this hypothesis in this PPAE mouse model (1), the brainstem gene expression of the serotonin transporter (the Solute Carrier Family 6 Member 4, SLC6A4), the BDNF, the NGF, the $TrkA^{NGF}$, the high affinity BDNF receptor ($TrkB^{BDNF}$), the receptor binding the endogenous neurotransmitter serotonin (5-Hydroxytryptamine Receptor 2C, HTR2C) and finally the p75 receptor in adult offspring were analyzed either in presence or in lack of postnatal alcohol exposure. We focused on the brainstem because the importance of brainstem in the modulation of large-scale intrinsic cortical activity as targets of the pharmacological treatment of major neurological and psychiatric disorders (64) and because serotonergic projections originate from the raphe nuclei in the brainstem (65). We predicted that PPAE may affect the neurotrophins routes of the postnatal sensitivity to alcohol drinking in adult offspring by inducing changes in the serotonergic tone.

2. MATERIALS AND METHODS

2.1 Animals

The mouse tissues used in the present investigation derive from the animals previously utilized for investigating alcohol sensitivity affected by PPAE (1). Briefly, CD1 outbred male mice were used in this experiment (1). All animals were 3 months old and housed singly at the beginning of the experiments in Plexiglas cages (33 × 13 × 14 cm) under standardized conditions with pellet food (enriched standard diet purchased from Mucedola, Settimo Milanese, Italy). Food (Purina Lab Chow #5015) and drinking solutions were available *ad libitum*. A 12L:12D lighting regime was used.

Eight male CD1 mice were randomly divided into two groups: a group of mice (n = 4) received *ad libitum*, as the only source of liquid, after a habituation period of 10 days, ethanol 11 percent dissolved in water for 60 days. The second group of mice (n = 4) received sucrose dissolved in water at equivalent caloric intake of the ethanol group and was used as control group. The ethanol used for the preparation of the drinking solution was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was of analytical grade. The daily consumption of ethanol solution was measured day by day. Two months after treatment, males were allowed to breed with non-treated females (n = 8), for 2 hours, without any source of liquid. The presence of a copulation plug in female mice was presumed to indicate mating. At birth, all litters were reduced to four males only. The pups remained with their own mother based on methods previously described (62). The experiments were carried out only on the male isolated offspring (15 offspring sired by control sires and 15 offspring sired by alcohol-exposed sires). To investigate ethanol consumption preference in the offspring, 60-day-old offspring were treated intraperitoneally (i.p.) with a single administration of ethanol (0.5 or 1.5 g/kg) or vehicle (saline solution) to generate six experimental groups, namely: PPAE-Veh; PPAE-0.5 EtOH; PPAE-1.5 EtOH; non-PPAE-Veh; non-PPAE-0.5 EtOH; non-PPAE-1.5 EtOH (n = 5 for each experimental group). All efforts were made to minimize and reduce animal suffering and for limiting the number of animals used. All animal experiments were carried out following the procedure described in the guidelines of the European Community Council Directive of 24 November 1986 (86/609/EEC).

2.2 Blood and brain tissue dissection

Offspring were sacrificed by a guillotine 7-days after the end of the behavioral experiments as explained in (1). Sires were sacrificed 3 days after the discovery of the vaginal plug of the dams. The blood was collected in heparin vials and quickly centrifuged at 10000 rpm for 15 minutes. The brain was quickly removed, the brainstem dissected out using a mouse brain matrix (ASI Instruments, Inc. Co., Warren, MI, USA) and stored at −80°C until used according to methods previously described (1, 61). The brainstem was then homogenized and centrifuged at 8500 rpm and the supernatant used for RT-PCR analysis.

2.3 Blood ethanol levels by gas chromatography/head space (HS) procedure

Gas chromatography/HS was applied in this research to determine blood alcohol concentration in whole blood samples. We used a Clarus 600 Gas Chromatography Perkin Elmer and a TurboMatrix 40 Trap HeadSpace Sampler Perkin Elmer with FID detector. Analytical conditions were set up and the method was validated by a previous study. 100 μl of whole blood was collected with a micropipette and transferred into a gas chromatography (GC) vial. The gasses that are formed inside GC vial was collected in GC to be analyzed. It is important to firmly close the vial to prevent the evaporation of ethanol during time and to conserve the vial inside of a refrigerator. Standard solutions were set up for calibration curve at 100, 50, 25, 12.5, 6.2 and 3.1 mg% of pure ethanol and were obtained by consequent dilutions of pure ethanol in distilled water.

2.4 RNA extraction and quantification

Brainstem total RNA was isolated by Trizol Reagent (Invit-

rogen Corporation Carlsbad, CA, USA). The quantification and purity of the RNA extract were evaluated by the use of a fluorometer (Qubit, Invitrogen) and spectrophotometric reading of absorbance at 260/280 nm.

2.5 RT-PCR and expression analysis

Deoxyribonuclease (DNase) treatment was carried out to remove residual amounts of contaminating genomic DNA: total RNA was incubated with 2.2 units of *DNase I* (New England Biolabs, Ipswich, MA, USA) at 37°C for 10 min in a final volume of 20-30 µl. Subsequently, the sample was treated with 1X EDTA (50 mM, pH = 8) at 75° C for 10 min, to deactivate DNase I. After digestion, 2 µg total RNA was reverse transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) following the manufacturer's specifications.

Real-time PCRs were carried out using iTaq Universal Probes Supermix (Bio-Rad, Hercules, CA, USA) and TaqMan gene expression assays (Applied Biosystem, Forster City, CA, USA), reported in Table 1, according to the suppliers' specification (50° C for 2min, 45 cycles at 95° C for 15min, 60° C for 1min) in a Bio-Rad Mini-Opticon system. Housekeeping genes Gadph and RNA 18s were used to ensure the validity and reproducibility of results. Data for each target gene were normalized with the endogenous reference gene, and the fold change in target gene abundance was determined using the $2^{-\Delta\Delta Ct}$ method.

Table 1. *TaqMan assays used to perform the Real-Time PCR*

GENE	Assay ID
SLC6A4	Mm_00439391_m1
BDNF	Mm_04230607_m1
NGF	Mm_00443039_m1
$TrkB^{BDNF}$	Mm_00435422_m1
$TrkA^{NGF}$	Mm_01219406_m1
$p75^{NTR}$	Mm_00446296_m1
HTR2C	Mm_00434127_m1
Gadph	Mm_99999915_g1

2.6 Statistical analysis

Data on offspring were analyzed by two-way ANOVAs considering as factors the paternal ethanol exposure and the ethanol treatment according to methods previously described (66, 67). Sires data were analyzed by the Student's t-test. Post-hoc comparisons within logical sets of means were performed by the Tukey's HSD test, the use of which is permissible or even recommended in the absence of significant main or interaction effects in the ANOVA to minimize frequency errors of both type I and type II as suggested by Wilcox (68).

3. RESULTS

3.1 PPAE mouse model description (1)

Ethanol consumption did not cause changes in the body weight of fathers; at the time of mating no differences in body weight between the ethanol group of sires and the sucrose group were found. Food and liquid consumption of ethanol-exposed fathers and control sires were also similar. In the sires of ethanol group, a cautious day-by-day analysis of liquid consumption showed ethanol consumption values comprised between 0.15 and 0.26 ml with a mean daily intake of 0.20 ± 0.018 ml. The blood ethanol levels in the ethanol-exposed fathers 1 day after mating, expressed as mg/100 ml of mouse blood, were between 3.5 and 21.5 mg/ 100 ml. As for the PPAE offspring, no modifications in numbers of pups (litter size), perinatal mortality, and numbers of dead-born pups were observed. Quite surprisingly, PPAE offspring had significant lesser values of body weight as revealed at the time of mouse sacrifice when compared with the body weight of controls offspring without any effects of ethanol administration ($p<0.01$ in the ANOVA for the effect of ethanol administration).

3.2 RT-PCR for SLC6A4 – BDNF – NGF -$TrkA^{NGF}$ – $TrkB^{BDNF}$ – HTR2C – $p75^{NTR}$ Brainstem Gene Expression

The data on the SLC6A4 gene coding the serotonin transporter also known as the sodium-dependent serotonin transporter and solute carrier family 6 member 4 are shown in Figure 1A. A main effect of paternal alcohol exposure was clearly disclosed by ANOVA with an increase [$p=0.01$] in PPAE animals due to the difference levels in gene expression between PPAE-Veh mice and non-PPAE-Veh ($p<0.05$ in post-hoc comparison). Data did not evidence other effects of the postnatal alcohol treatment or of the interaction between PPAE and postnatal alcohol treatment.

Data on the HTR2C receptor expression in the mouse brainstem, a subtype of 5-HT receptor binding the endogenous neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) are shown in the panel B of the Figure 1. ANOVA clearly showed an interaction paternal pre-conceptual alcohol exposure x postnatal alcohol treatment [$p<0.05$]. Definitely, post-hocs evidenced high HTR2C expression in PPAE mice exposed postnatally to

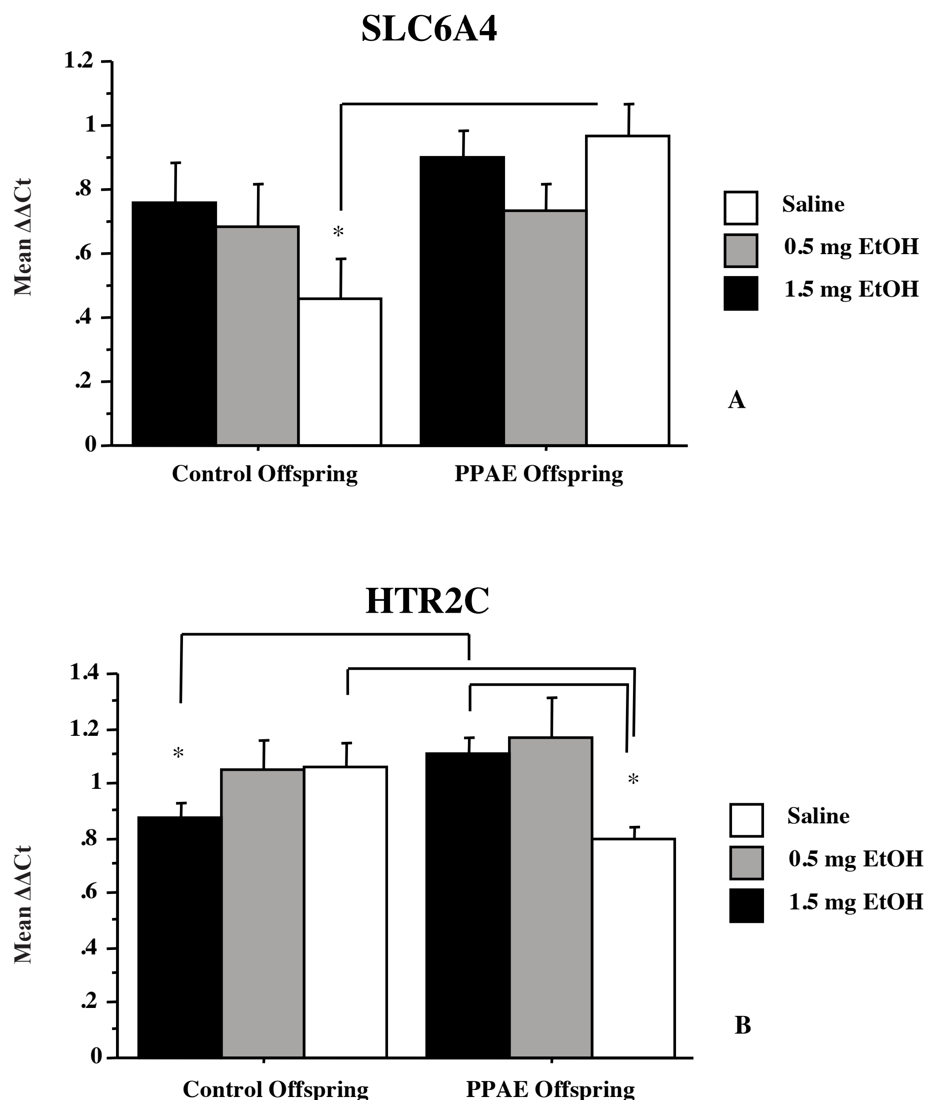

Figure 1. *Normalized expression (ΔΔCt) of the SLC6A4 gene (panel A) and the HTR2C gene (panel B). Each column represents the mean of expressions of individuals belonging to a certain experimental condition: PPAE offspring saline; PPAE offspring 0.5 mg/kg EtOH; PPAE offspring 1.5 mg/kg EtOH; Control offspring saline; Control offspring 0.5 mg/kg EtOH; Control offspring 1.5 mg/kg EtOH. See Methods. The error bars indicate pooled standard error means (SEM) derived from appropriate error mean square in the ANOVA. Asterisks indicate significant differences between groups (*, p < 0.05).*

alcohol if compared with PPAE-Veh or non-PPAE-1.5 EtOH animals (ps<0.05). Post-hocs also revealed differences between PPAE-Veh and non-PPAE-Veh groups with lower expression in PPAE-Veh mice (p<0.05).

Figure 2A shows the results of the BDNF expression in the brainstem of mice paternally and postnatally exposed to alcohol. ANOVA clearly disclosed an effect of the postnatal alcohol treatment [p<0.05] with low expression levels in the 0.5 EtOH groups but particularly in the offspring of non-PPAE-0.5 EtOH (p<0.05 in post-hocs). Data of $TrkB^{BDNF}$ expression are shown in Fig. 3A. A main effect of the postnatal alcohol treatment was revealed by ANOVA (p<0.01) with an elevation in the 0.5 EtOH groups. In particular, post-hoc comparisons disclosed a potentiation in $TrkB^{BDNF}$ expression in PPAE-0.5 EtOH if matched with both PPAE-1.5 EtOH and PPAE-Veh (ps<0.05).

Surprisingly, Figures 2B and 3B show no differences between groups for the NGF and $TrkA^{NGF}$ expression, respectively. Figure 3C shows the ANOVA expression data of the $p75^{NTR}$

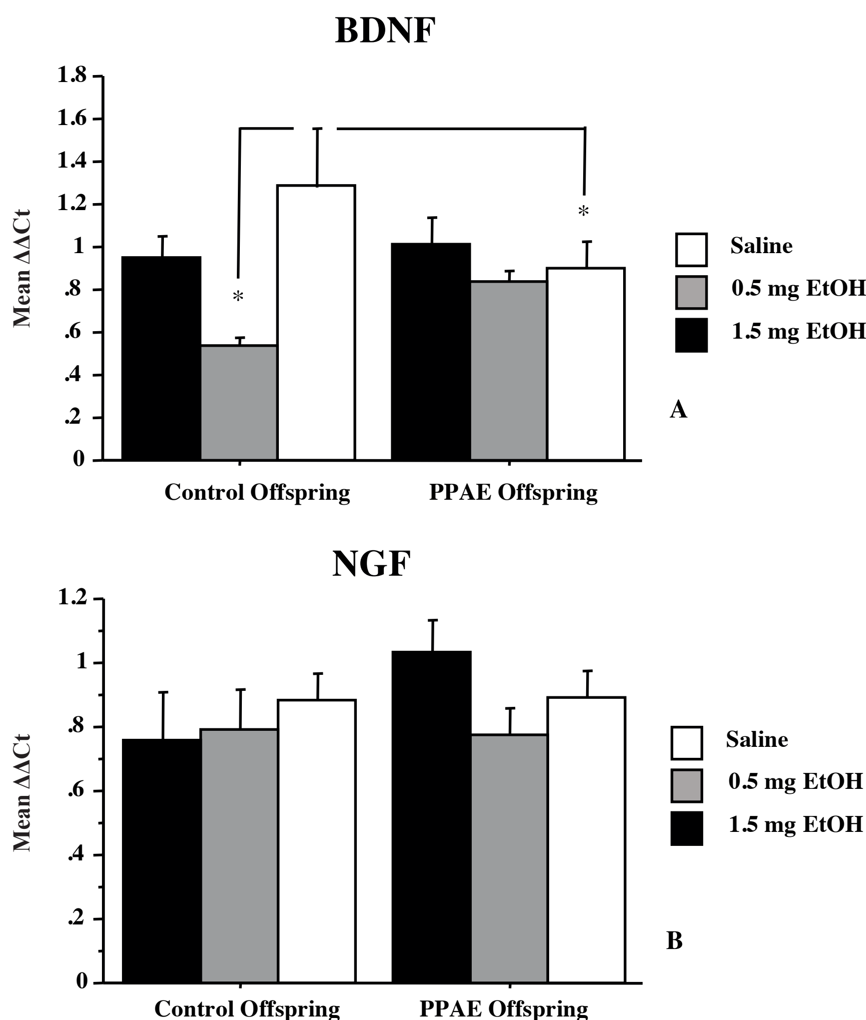

Figure 2. *Normalized expression (ΔΔCt) of the BDNF gene (panel A) and the NGF gene (panel B). Each column represents the mean of expressions of individuals belonging to a certain experimental condition: PPAE offspring saline; PPAE offspring 0.5 mg/kg EtOH; PPAE offspring 1.5 mg/kg EtOH; Control offspring saline; Control offspring 0.5 mg/kg EtOH; Control offspring 1.5 mg/kg EtOH. See Methods. The error bars indicate pooled standard error means (SEM) derived from appropriate error mean square in the ANOVA. Asterisks indicate significant differences between groups (*, p < 0.05).*

known to bind all neurotrophins. Indeed, ANOVA disclosed a main effect of the paternal alcohol exposure ($p<0.05$) and also a main effect of the postnatal alcohol exposure ($p=0.05$). Post-hoc comparison showed higher expression in both PPAE-Veh and PPAE-0.5 EtOH animals when opposed to PPAE-1.5 EtOH mice ($ps<0.05$). Furthermore, $p75^{NTR}$ expression in PPAE-Veh mice was also elevated if compared with p75 expression in non-PPAE-Veh animals ($p<0.05$).

The panels of Figure 4 show the SLC6A4, BDNF, NGF, $TrkA^{NGF}$, $TrkB^{BDNF}$, HTR2C and $p75^{NTR}$ mouse brainstem gene expression in both chronic exposed sires and non-exposed sires. Student's t-test data did not reveal differences between the two groups of animals.

4. DISCUSSION

In this study we used a PPAE mouse model to investigate SLC6A4, HTR2C, $TrkA^{NGF}$, $TrkB^{BDNF}$, $p75^{NTR}$, BDNF and NGF gene expression at the brainstem level, and thus get more insights about the mechanisms underlying alcohol drinking behavior and the neurobiological sensitivity to alcohol consumption that could be disrupted in the offspring by altered heritability of pathways regulating mood and serotonergic

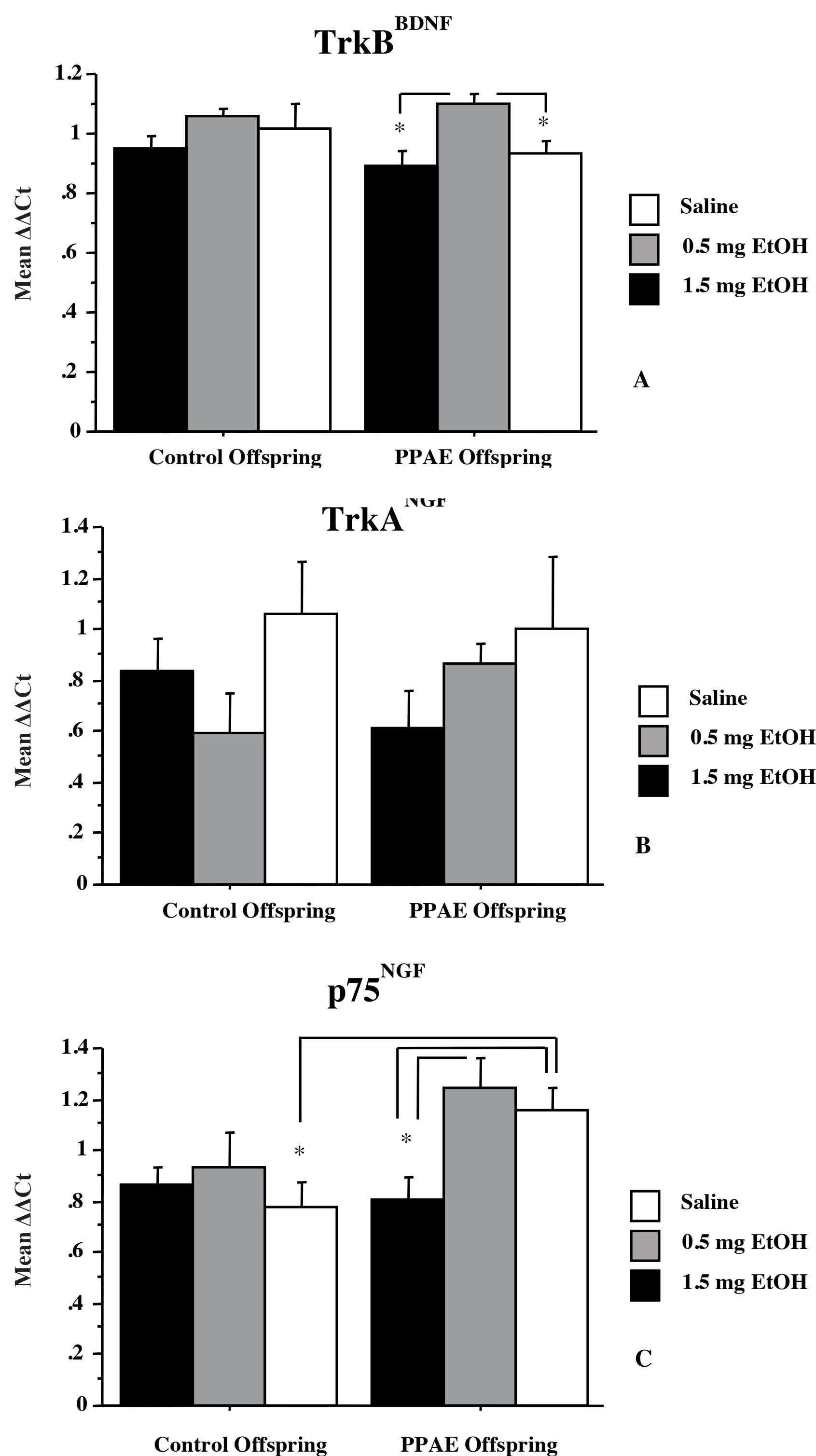

Figure 3. *Normalized expression (ΔΔCt) of the $TrkB^{BDNF}$ gene (panel A), the $TrkA^{NGF}$ gene (panel B) and $p75^{NTR}$ gene (panel C). Each column represents the mean of expressions of individuals belonging to a certain experimental condition: PPAE offspring saline; PPAE offspring 0.5 mg/kg EtOH; PPAE offspring 1.5 mg/kg EtOH; Control offspring saline; Control offspring 0.5 mg/kg EtOH; Control offspring 1.5 mg/kg EtOH. See Methods. The error bars indicate pooled standard error means (SEM) derived from appropriate error mean square in the ANOVA. Asterisks indicate significant differences between groups (*, $p < 0.05$). PPAE = pre-conceptual paternal alcohol exposure.*

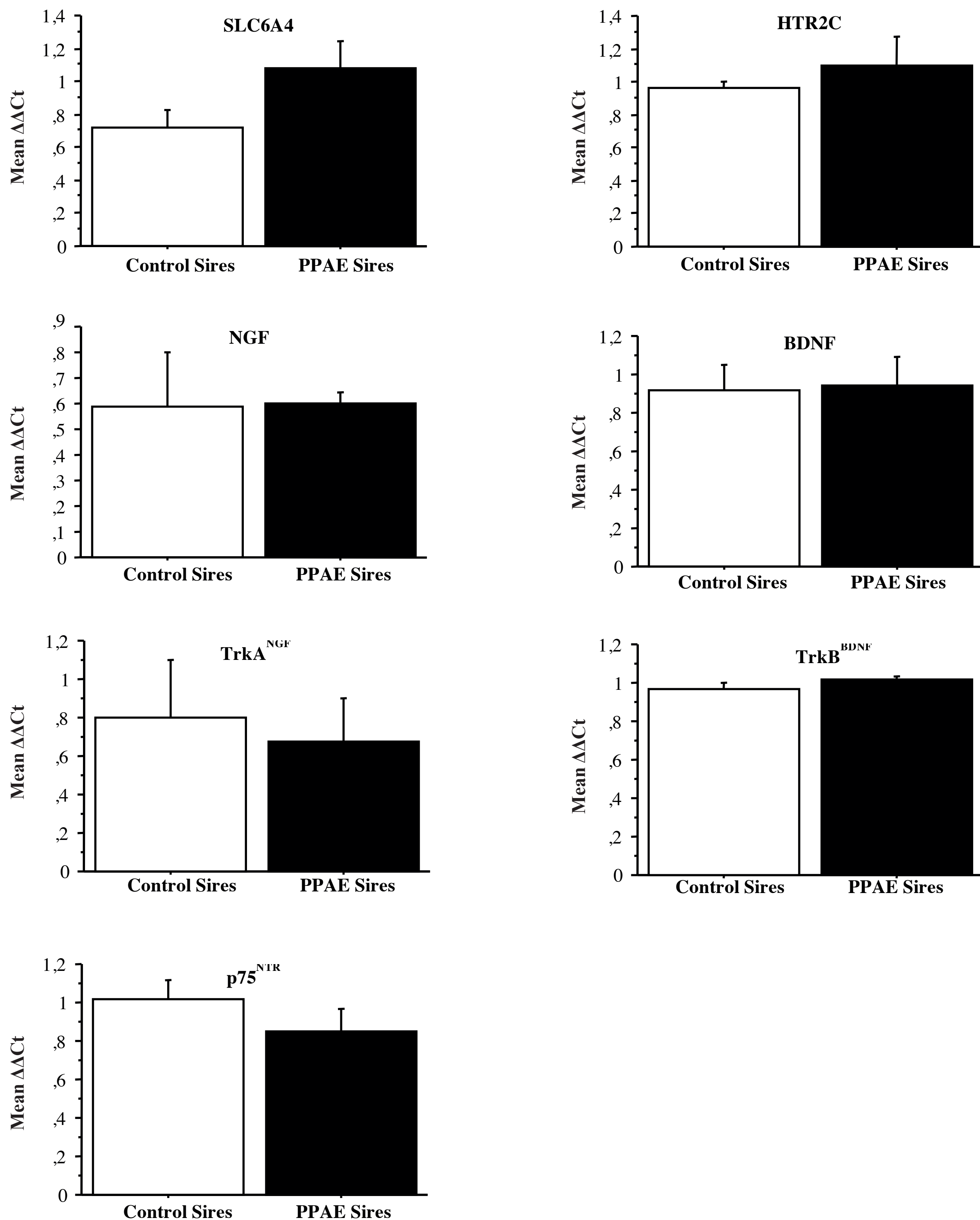

Figure 4. *Normalized expression (ΔΔCt) of the SCL6A4, HTR2C BDNF, NGF, TrkB^{BDNF}, TrkA^{NGF} and p75^{NTR} genes. Each column represents the mean of expressions of individuals belonging to a certain experimental condition: PPAE sires and Control sires. See Methods. The error bars indicate pooled standard error means (SEM) derived from appropriate error mean square in the Student' t-test.*

status via the changes in the expression of neurotrophins such as NGF and BDNF.

Considering the gene expression of proteins involved in brain serotonin functions, we found (i) the potentiation of SLC6A4 expression in the offspring of chronic alcohol dependent sires (i.e., an increase in a monoamine transporter protein that transports serotonin from the synaptic cleft back to the presynaptic neuron), and (ii) a decrease in HTR2C gene expression (i.e., 2C 5-HT receptor subtype) in the offspring of chronic alcohol dependent sires. The occurrence of mood disorders is known to be influenced by both genetic and environmental factors. However, it is becoming clear that few genetic and environmental factors can act in concert to regulate susceptibility to psychiatric or addictive disorders (69). Serotonergic projections originate from the raphe nuclei in the brainstem and mouse models of defective serotonergic transmission have proven to be powerful tools for the understanding the contribution of serotonin in the regulation of emotion (70). Recent reviews on role of serotonin transporter and serotonin receptors in alcohol dependence and RNA editing of 5-HT2C receptors and alcohol intake provides a clue not only to the development of alcohol dependence with chronic exposure, it also implies that induced editing alterations may occur trans-generationally (71, 72). Indeed, changes in SLC6A4 transporter and HTR2C have been extensively examined as potential AUD risk factor (73–75), and differences in humans SLC6A4 clinical phenotype are considered a key target for AUD pharmacological interventions (76). Furthermore, the activation of HTR2C by serotonin (77, 78) may inhibit dopamine and norepinephrine release in specific brain areas (79). Interestingly, it has been shown that some suicide victims have an abnormally high number of HTR2C receptors in the prefrontal cortex (80) and the pharmacological blockade of HTR2C may increase both dopamine and norepinephrine activity in the frontal cortex. Conversely, many selective serotonin reuptake inhibitors (SSRIs) indirectly stimulate HTR2C activity by increasing the serotonin levels in the synaptic cleft, although the mood elevation that is usually observed after SSRIs treatment is usually paralleled by the downregulation of the HTR2C (81, 82). 5-HT2C receptors mediate the presence of extracellular dopamine in response to many drugs, including nicotine, amphetamine, morphine and cocaine (79). HTR2C antagonism modulates dopamine release in response to reinforcing drugs and many dopamine-eliciting stimuli. Rewarding feeding, social interaction and sexual activity all release dopamine as result of HTR2C inhibition. By contrast, the increase in HTR2C expression reduces dopamine release in a stimulus-independent manner (79).

Interestingly, increased genetic susceptibility to depression has been observed in population studies from subjects carrying polymorphism of the serotonin transporter (5-HTT) in the SLC6A4 gene (5-HTT-linked polymorphic region, 5-HTTLPR), whose alterations are considered of key relevance for depression pathophysiology (83, 84). The 5-HTT is also a well-known major molecular target of the SSRI class of drugs, and consequently the most reliable factor to predict antidepressant efficacy (85). From this view, it is tempting to consider the potential relationship between the increase in SLC6A4 expression that we found in the offspring of alcohol-exposed sires and both latent susceptibilities to develop depression and refractivity to SSRI treatment. Moreover, the concomitant decrease that we found in HTR2C gene expression suggests the possibility that the downregulation of the 5-HT2C receptor might contribute to exacerbate dopamine release in response to alcohol intake and account for the enhanced sensitivity to alcohol rewarding effects observed in the offspring of alcoholic sires (1).

As for the gene expression of neurotrophins playing crucial roles also in the emotion regulation and development including mood disorders, we found changes in BDNF and in both its high and low affinity receptors, $TrkB^{BDNF}$ and $p75^{NTR}$, respectively. In particular, a $p75^{NTR}$ gene expression elevation was found in the brainstem in the offspring of chronic addicted sires. We also previously found a postnatal sensitization (1) to low alcohol doses in the offspring from chronic exposed sires associated with changes in $TrkB^{BDNF}$ and BDNF expression, whereas no changes were found for both NGF and $TrkA^{NGF}$. In humans, mood disorders (unipolar and bipolar depression) are complex diseases characterized by impaired mood, anhedonia, anxiety, neurovegetative symptoms and cognitive damage. Mood disorders are characterized by a reduction in neurogenesis, alteration in synaptic structure and synaptic transmission, all of them controlled by BDNF, a neurotrophin performing multiple functions in the adult central nervous system (86). Growing evidence show that BDNF is critically decreased in mood disorders and plays an essential role in most of anti-depressant treatments (86). In particular, the BDNF levels are reduced in the brain and serum of depressed patients and at least the reduction in serum levels is reversible upon successful pharmacological treatment (87). These data, together with an abundance of reports using several animal models with depression-like behavior or control of expression

of BDNF or its receptor $TrkB^{BDNF}$ have associated BDNF in the pathophysiology of depression as well as in the mechanism of action of antidepressants (86, 87). Recent data have shown that the post-translational processing of BDNF gene outcome can produce different molecular units that differently regulate signaling through $TrkB^{BDNF}$ and $p75^{NTR}$. Aberrant neuroadaptation of BDNF signaling and alterations in synapse activity/plasticity have been associated with the pathophysiology of AUD and stress-related disorders (88, 89). In particular, the complex interaction between BDNF and alcohol provides evidence to suggest that corticostriatal BDNF signaling acts to keep alcohol drinking in moderation (90). Rodent models indicate that moderate alcohol consumption increases BDNF levels in the brain and suggest also that alcohol intake levels escalate when the endogenous corticostriatal BDNF pathway becomes dysregulated (91). Interestingly, a recent human study on BDNF, $p75^{NTR}$ and $TrkB^{BDNF}$ mRNA expression in addicted patients demonstrated that $p75^{NTR}$ levels were slightly increased while BDNF and $TrkB^{BDNF}$ were significantly decreased leading to the hypothesis that the balance between the BDNF receptors signaling pathways appeared dysregulated in alcohol dependence (92). In particular, under the present experimental conditions, the potentiation in $TrkB^{BDNF}$ expression was observed following postnatal low dose alcohol administration, and particularly in the offspring of exposed sires where appears to correlate with the alcohol pexpreference/rewarding properties we observed in a previous study (1). Indeed, we demonstrated that PPAE elicited a significant susceptibility to the alcohol rewarding effects mainly evident at a low concentration (0.5 g/kg) that was ineffective in non-PPAE offspring (1). We also found that, higher alcohol dosage (1.5 g/kg) induced an aversive response in PPAE animals while elicited a significant preference in non-PPAE offspring (1). In the present study no changes were observed in the expression levels of NGF and its receptor in the offspring of the ethanol-exposed sires (cf. 1). Also, the two studies also find opposite changes in BDNF expression. In a previous study, offspring of exposed male mice to chronic vapor ethanol or control conditions had reduced ethanol preference and consumption, enhanced sensitivity to the anxiolytic and motor-enhancing effects of ethanol, and increased BDNF expression in the ventral tegmental area (35). In particular, ethanol exposure also decreased DNA methylation at the BDNF promoter of sire's germ cells and hypomethylation was maintained in the VTA of both male and female ethanol-sired offspring (35).

Another finding of the present investigation was the absence of effect of the chronic ethanol exposure in the sires on the investigated parameters that might be interpreted as a lack of dependence in the sires that were forced to drink ethanol (93). As for the correlation BDNF/5-HT systems _it is known that BDNF and 5-HT possess 2 distinct signaling systems that play regulatory roles in many neuronal functions including survival, neurogenesis, and synaptic plasticity. A common feature of the two systems is their ability to regulate the development and plasticity of neural circuits involved in mood disorders such as depression and anxiety. BDNF promotes the survival and differentiation of 5-HT neurons. Conversely, administration of antidepressant selective serotonin reuptake inhibitors enhances BDNF gene expression. There is also evidence for synergism between the two systems in affective behaviors and genetic epitasis between BDNF and the serotonin transporter genes (94). Furthermore, recent findings on the interaction between BDNF and 5-HT systems due to long-term ethanol consumption in mice showed considerable impact of alcoholization on the BDNF system, resulting in proBDNF and $p75^{NTR}$ receptor expression enhancement (95). Such changes were mostly present in the raphe nuclei area where the majority of the cell bodies of the 5-HT neurons are localized, as well as in the cortex, hippocampus, and amygdala suggesting that the BDNF/5-HT interaction may contribute to the mechanism underlying chronic alcohol-induced neurodegenerative disorders (95).

5. CONCLUSION

The combined data from the PPAE present and parent (1) studies demonstrate that (i) the PPAE mouse model here described is characterized by a higher postnatally sensitivity to the effects of alcohol exposure, (ii) in this PPAE mouse model, where genotype effects can be carefully measured, we disclosed that exposure of sires to alcohol prior to mating may disrupt the sensitivity to the serotonergic-associated effects of alcohol, thus influencing the post-natal alcohol preference in the offspring, and (iii) alcohol drinking behavior and the sensitivity to the behavioral effects of alcohol may be epigenetically transmitted through the male lineage. Furthermore, if such kind of animal model studies could be applied to the effects of alcohol abuse in humans, these findings may contribute *to raise public concern* in consideration of the large percentage of men drinking alcohol prior to procreation. The data of the present study may represent a further step in the attempt to unravel some genetic events involved in paternal alcohol consumption and, likewise, this study may draw significant interests among those working on PPAE/FASD animal models.

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DISCLOSURE STATEMENT

Nothing to disclose

CONFLICTS OF INTEREST

All the authors do declare no conflicts of interest.

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